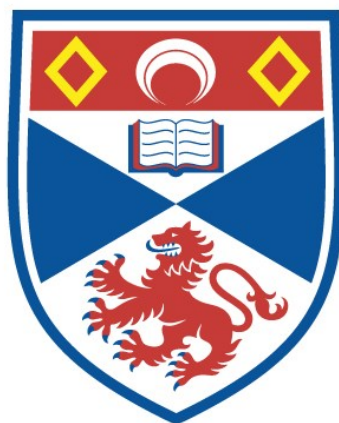


BENZIMIDAZOLE NUCLEOSIDE ANALOGUES AS  
POTENTIAL ANTIHERPETIC AGENTS

Michel W. Ritchie

A Thesis Submitted for the Degree of PhD  
at the  
University of St Andrews



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# Benzimidazole Nucleoside Analogues as Potential Antiherpetic Agents

A thesis by

Michael W. Ritchie



submitted for the degree of Doctor of Philosophy in the  
Faculty of Science of the University of St. Andrews

September 1996

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I hereby certify that Michael William Ritchie has fulfilled the Regulations appropriate to the Degree of Ph.D.

September 1996

Signed.....

## Dedication

This thesis is dedicated to my Mum, Dad and Sister.

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I would like to express my gratitude to Dr. David M. Smith for getting me to this stage through his supervision, endless help and encouragement.

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Thanks are also due to the following staff in the School of Chemistry: Mrs. M. Smith for high-field  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra, Mr. C. Millar for mass spectra and Mrs. S. Williamson for the C/H/N analyses.

Many thanks are also due to Professor R.T. Hay and Mr. D.F. Young of the School of Biological and Medical Sciences for their help with the assay design and antiviral testing of the benzimidazole nucleosides synthesised. Thanks are also extended to Dr. R.J. Young of the GlaxoWellcome Research Centre, Stevenage for many helpful suggestions and for coordination of the external antiviral testing.

Finally, I would like to thank all the people with whom I have had the fortune to share a lab over the past few years for their good company, stimulating (!) conversation and endless distractions.

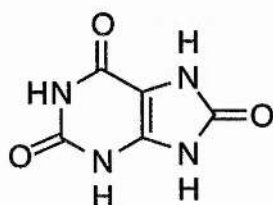
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## Chapter 1

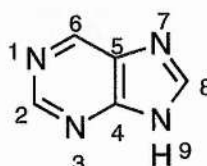
### Introduction

#### 1.1 The history of nucleic acids and nucleoside analogues

The discovery of nitrogen containing heterocyclic compounds featured in the early development of "organic" chemistry which concentrated on the study of materials isolated from living sources. Uric acid (1), a purine derivative, was one of those early discoveries, having been isolated from gall-stones and urine by Scheele<sup>1</sup> in 1776 but the elucidation of the correct structure of this purine did not occur for a further 100 years.

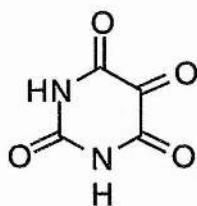


Uric Acid (1)

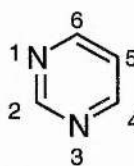


Purine numbering (2)

Alloxan (3), the first pyrimidine, was isolated in 1818 by Brugnatelli<sup>2</sup> via the oxidation of uric acid with nitric acid.



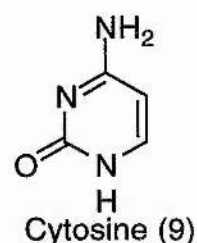
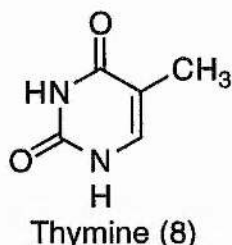
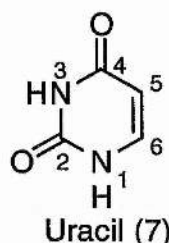
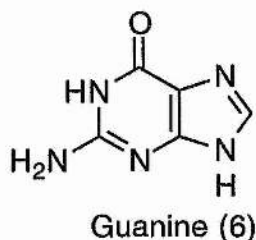
Alloxan (3)



Pyrimidine numbering (4)

Of the five common nucleoside bases, the most common purines, adenine (5) and guanine (6), have been known since 1885 and 1884 respectively<sup>3</sup>, although other bases have been isolated more recently from RNA including 2-methyl-6-aminopurine, 6-

methylaminopurine, 6-(N,N-dimethylamino)purine, 6-hydroxy-2-methylaminopurine and 1-methylguanine<sup>4</sup>. The three common members of the pyrimidine group comprise uracil (7), thymine (8), and cytosine (9). Another minor pyrimidine component is 5-methylcytosine<sup>5</sup>.



Although certain purines and pyrimidines had been isolated from natural sources at earlier dates and the first nucleoside, inosinic acid, was discovered by Liebig in 1847<sup>6</sup>, nucleic acid research really began with Miescher's<sup>7</sup> isolation of a material with a high phosphorus content which he termed *nuclein*. The term *nucleic acid* was coined by Altmann<sup>8</sup> who continued Miescher's work after the death of the latter. By 1914 the work of Levene<sup>9</sup> had determined all the main constituent bases of the nucleic acids as well as the sugar D-ribose. The existence of two distinct types of nucleic acid had also become apparent with Levene and Bass<sup>10</sup> isolating 2-deoxy-D-ribose at a later date. The first nucleosides to be synthesised were the glucose analogues of the natural purine and pyrimidine nucleosides by Hilbert and Johnson<sup>11</sup> but Todd and co-workers<sup>12</sup> in Cambridge synthesised the first natural nucleosides using a similar synthesis and continued with these rigorous syntheses and proofs of structure of further nucleosides and nucleotides. Thus by the 1940s all the major components of the nucleic acids were known,

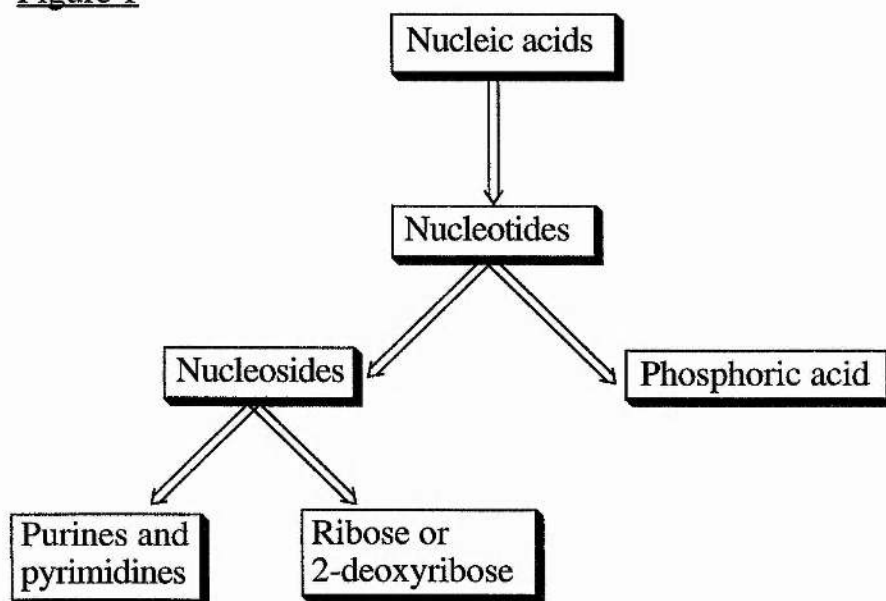


the  $\beta$ -configuration of nucleosides and the 3',5'-phosphodiester linkage had both been proposed but further progress did not occur until the introduction of more sensitive chromatographic techniques such as paper chromatography, paper electrophoresis and ion-exchange chromatography which allowed great improvement in the separation and purification of nucleosides, nucleotides and cellular components.

The careful analytical work undertaken by Chargaff<sup>13</sup> determined the base-pair ratio of adenine:thymine and guanine:cytosine and with the demonstration of the 3',5'-phosphodiester linkage by Brown and Todd<sup>14</sup>, the way was left open for the elucidation of the double helical structure of DNA by Watson and Crick<sup>15</sup> in 1953. Allied with the discovery in 1944 by Avery, MacLeod and McCarthy<sup>16</sup> that the genetic structure of cells was connected to DNA, the way in which genetic information was stored and inherited could now be fully understood and its importance fully appreciated.

Nucleic acids may be broken down to their building blocks by a series of successive hydrolyses to yield eventually the bases and sugars. This information is summarised in figure 1.

Figure 1



The term *nucleoside*, originally devised by Levene and Jacob<sup>17</sup> and restricted to the

purine and pyrimidine *N*-glycosides of ribose and 2-deoxyribose, is now more generally applied to other heterocyclic glycosides, including the 5,6-dimethylbenzimidazole riboside of vitamin B<sub>12</sub> and all other carbohydrate derivatives of *N*-heterocyclic compounds irrespective of attachment through nitrogen, carbon or oxygen. A nucleoside (in terms of DNA or RNA) is normally a  $\beta$ -glycoside consisting of a base (purine or pyrimidine) bonded via an *N*-glycosyl linkage to a sugar (ribose or 2-deoxyribose). The bond is formed between N-9 of a purine or N-1 of a pyrimidine to C-1' of either of the aforementioned sugars.

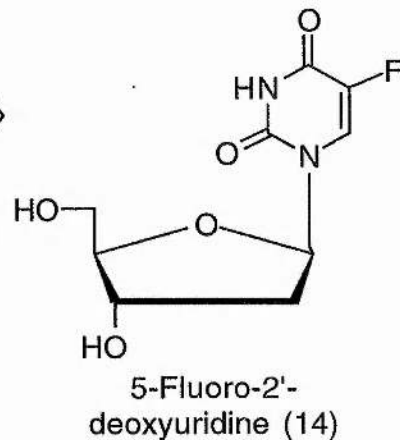
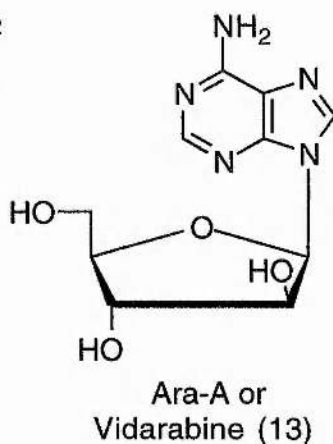
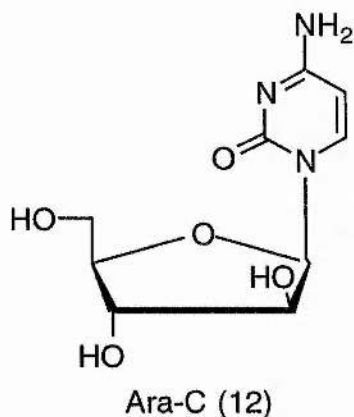
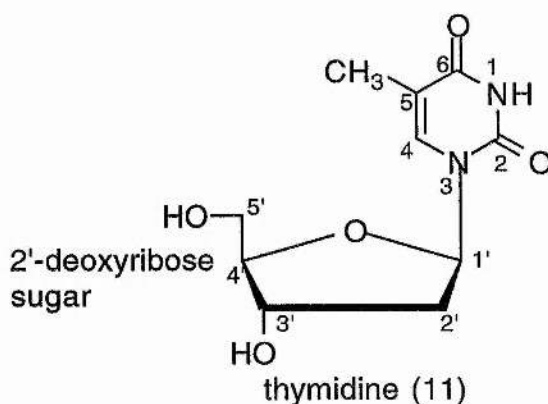
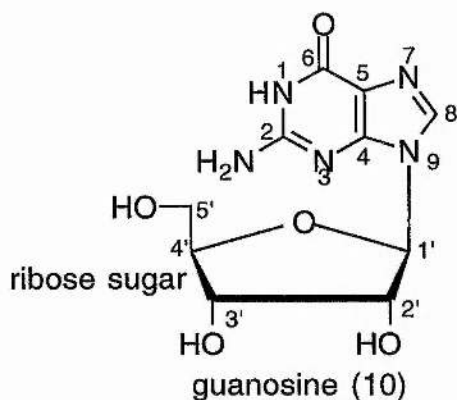
The term *nucleotide* was also devised by Levene<sup>18</sup> and is the phosphate monoester of the nucleoside, formally derived by the reaction of the 5'-hydroxyl of the sugar and phosphoric acid; the phosphate monoesters may also be formed on any of the other hydroxyl groups. Cyclic phosphates may be formed between any two of the hydroxyl groups; di- and tri-phosphates on the 5'-hydroxyl also exist in the cellular environment.

The biosynthesis of nucleic acids involves polymerisation of nucleoside triphosphates, with the concomitant release of pyrophosphate. The 5'-hydroxyl of one nucleotide is linked with the free 3'-hydroxyl of another giving an alternating sugar-phosphate backbone with a 3',5'-phosphodiester linkage. The pyrophosphate combines with another nucleotide to maintain the cellular energy level. In the case of DNA, complementary bases pair up through intermolecular hydrogen bonding to give complementary strands where adenine bonds to thymine and guanine bonds to cytosine resulting in the formation of a double helix. In RNA uracil replaces thymine, and bonds to adenine if a double helix is present. In the cellular environment these processes are aided by enzymes, coenzymes and very many other essential components.

## 1.2 Some early antiviral drugs

Some antiviral drugs (nucleoside analogues) work by mimicking the natural nucleoside and are incorporated into the biochemical pathways of the virally infected cell, disrupting the normal processes and hence resulting in cell death and the prevention of

proliferation of the virus. For the nucleoside analogue to be accepted in place of a natural nucleoside it must have a very similar structure to the usual substrate: hence antiviral chemotherapy is an extremely new science resulting from recent advances in the understanding of the structures of DNA and RNA.

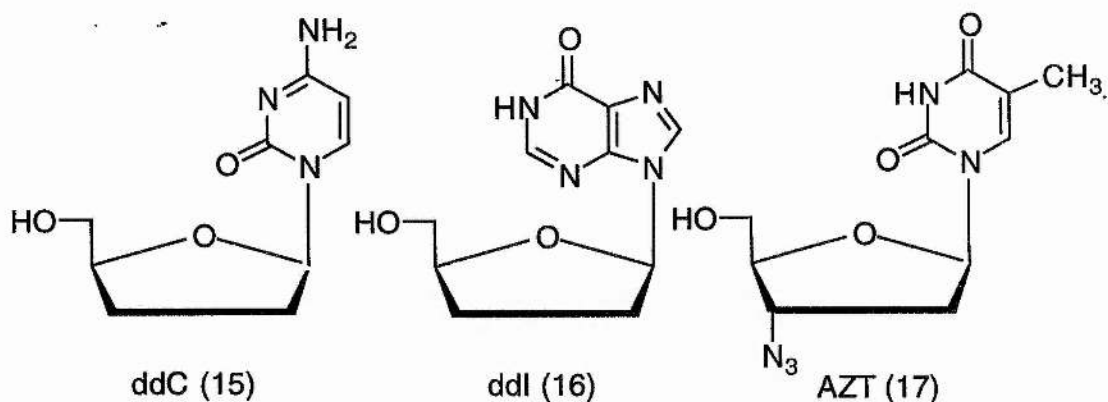


Natural nucleosides and unnatural analogues have been studied widely in a search for antiviral, fungicidal and anticancer agents. Anticancer activity is displayed by 5-fluoro-2'-deoxyuridine (14)<sup>19</sup> and both 1- $\beta$ -D-arabinofuranosylcytosine (Ara-C, 12) and 9- $\beta$ -D-arabinofuranosyladenosine (Ara-A, Vidarabine, 13) also possess antiviral activity as well as anticancer activity<sup>20</sup> by interfering with nucleic acid biosynthesis. These display little selectivity between infected and host cell, the lack of selectivity being the root of associated side effects. Vidarabine (13) is phosphorylated by both cellular and viral thymidine kinases but its "selectivity" comes at the DNA polymerase level where the virally induced polymerase is 6-12 times more sensitive to vidarabine triphosphate. Before the discovery of

acyclovir (see page 9), vidarabine was the principal treatment for herpes simplex and varicella zoster viral infections.

### 1.3 HIV, AIDS and AZT

With the discovery of the HIV virus and AIDS as a serious illness with the potential to infect the population at large there has been an explosion of research into antiviral nucleoside analogues. The finding that the dideoxy nucleoside, AZT (3'-azido-3'-deoxythymidine, 17)<sup>21</sup> was a potential therapeutic agent for the treatment of AIDS (although initially developed as an anticancer therapy) and that ddI (2',3'-dideoxyinosine, 16)<sup>22</sup> and ddC (2',3'-dideoxycytidine, 15)<sup>22</sup> were the only other FDA-licensed drugs for this purpose has prompted further nucleoside analogue research. Each of the aforementioned drugs has a very similar mode of action. Each being phosphorylated by host cell enzymes, their triphosphates inhibit HIV reverse transcriptase (HIV RT) due to the absence of a 3'-hydroxyl for chain elongation hence stopping DNA polymerisation. HIV RT is 100 times more sensitive to AZT triphosphate (AZTTP) than DNA polymerase affording a high level of selectivity. DdI is converted intracellularly to dideoxyadenosine triphosphate, its active form, and so is suitable for those unresponsive to AZT therapy. DdC, on the other hand, is used in conjunction with AZT. In summary, AZT, ddI and ddC are prodrugs and are sequentially phosphorylated by thymidine kinase to the 5'-triphosphate which is then incorporated into the growing viral DNA chain and acts as a chain terminator, there being no 3'-hydroxyl group in any of these. Unfortunately, several side effects are associated with long term administration of the drugs and resistance has also been known to develop in the virus<sup>23</sup>. Trials have addressed the use of lower doses of AZT to lessen toxicity: these have proved promising with no difference being observed in the progression of opportunistic infections<sup>24</sup>.



#### 1.4 The herpes viruses and early anti-herpetic agents

Antiviral chemotherapy continues to be a very active research area with the major target being AIDS. However a recent change of direction away from nucleoside analogues towards protease inhibitors and non-nucleoside reverse transcriptase inhibitors has occurred. The mainstay of nucleoside analogue research – still the second largest area of antiviral research – is thus directed towards the herpes group of viruses.

The group of human herpes viruses may be divided into three different subfamilies based upon different viral characteristics, although the entire group have the ability, once initial infection has subsided, to remain latent and recur at any time on production of the correct stimulus.

The first subfamily includes herpes simplex virus types 1 and 2 (HSV1 and HSV2) and varicella zoster virus (VZV). In general terms, these affect the skin and the latent infection resides in the nervous system. HSV1 was the first human virus to be recognised. Among other infections, HSV1 is the cause of cold sores and HSV2 the cause of genital herpes but each is also responsible for conditions such as meningitis and encephalitis. The divisions between the infection are not rigid, as HSV2 may cause cold sores and HSV1 genital herpes. VZV manifests itself as chickenpox (varicella) and with recurrence of the latent infection in later life causes shingles (herpes zoster).

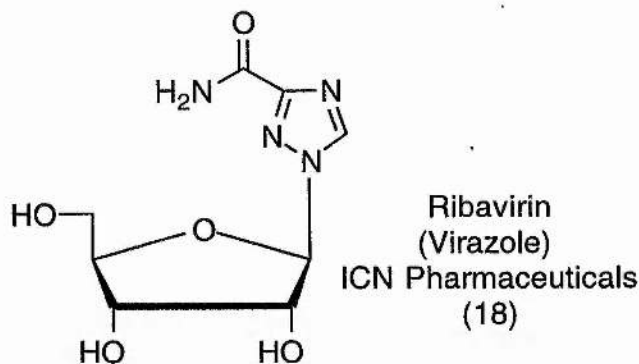
The second subfamily includes human herpes viruses 6 and 7 (HHV6 and HHV7)

which were both isolated recently from the blood of AIDS patients where the latent infection resides in particular types of blood cells. The third member of this subfamily is human cytomegalovirus (HCMV), which is present in 2-5% of newborn babies and up to 50% of adults in the developed world, the infection is usually asymptomatic. Reactivation of the latent phase is brought about by immunosuppression (artificial or through HIV infection) and is responsible for retinitis, pneumonia and other opportunistic infections.

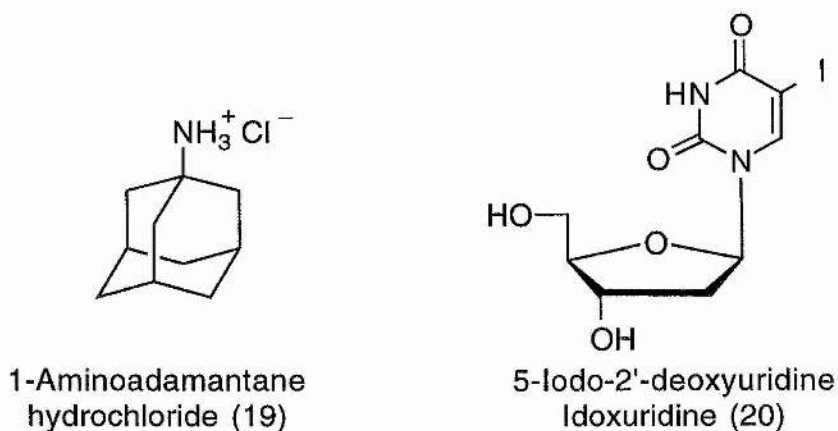
The final subfamily has only one member in Epstein Barr virus (EBV), the cause of glandular fever, for which there is no effective treatment apart from preferred exposure in early life when the infection is less severe. The herpes group of viruses is only one of a number of viral infections which cause serious conditions in the human and of particular concern are the effects these have on the immunocompromised.

Viral infections are very important economically in being the cause of the most days of absenteeism among the working population. As a virus is so small and simple in comparison to a bacterium, the possible points of attack for a therapeutic agent, where a difference in biochemistry may be pinpointed, are very few; the problems of producing a non-toxic, effective antiviral agent which is not harmful to the host are considerable. The common cold and influenza present little problem as recovery is almost always guaranteed, however many of the opportunistic viral infections which affect the immunosuppressed require extensive and effective treatment. The aim of antiviral chemotherapy is to suppress viral replication in infected cells with no or minimal damage to uninfected host cells. Selective inhibition of viral replication depends upon blocking one or more virus-specific metabolic steps in infected cells.

Of the more serious viral infections of the herpes group, ocular herpes keratitis and herpes virus encephalitis have proved amenable to antiviral chemotherapy. Ribavirin (Virazole, 18)<sup>25</sup>, a guanosine analogue in which the imidazole is replaced by a 1,2,4-triazole and the pyrimidine ring is not closed, was marketed by ICN Pharmaceuticals in some South American countries as an antiviral agent for treatment of these infections; it has multiple sites of action inhibiting many important viral processes and is also effective against Lassa fever.



Much effort has been expended in the search for effective antivirals with many other nucleoside analogues incorporating purine or pyrimidine bases and a variety of sugars showing activity against viruses. Some of the clinically useful compounds have included: 5-iodo-2'-deoxyuridine (Idoxuridine, 20)<sup>27</sup>, the first anti-HSV drug approved for human use, a mimic of thymidine which inhibits the biosynthesis of the latter and causes genome misreading and mutation resulting in virus inactivation; 9- $\beta$ -D-arabinofuranosyladenine (Vidarabine, Ara-A, 13, see pg. 5)<sup>20, 28</sup>; and some non-nucleoside antivirals including 1-aminoadamantane hydrochloride<sup>26</sup> (Amantadine, 19) which has activity against influenza A virus.



### 1.5 Acyclovir

Most significant progress was made with the drug acyclovir (Zovirax, 21, page



11)<sup>29</sup> (formerly produced by Wellcome, now GlaxoWellcome) which was regarded as the prototype of a totally new class of compounds with excellent and selective antiviral activity. This compound confirmed previous predictions that an intact sugar was not necessary for a nucleoside to bind to relevant enzymes<sup>30</sup>. Acyclovir has good activity against HSV1 and HSV2 with good selectivity over the host cell and virtually no cytotoxicity at therapeutic levels. Preliminary studies had also shown activity against VZV, but the activity is much lower and requires intravenous administration to achieve high plasma levels for effectiveness. Acyclovir has poor activity against HCMV (which does not code a thymidine kinase) and no activity against a range of RNA viruses<sup>31</sup> or HSV strains deficient in the ability to encode a thymidine kinase or DNA polymerase<sup>33</sup>.

**Table 1.1 Relative potencies of selected HSV1 treatments<sup>29</sup>**

<u>Compound</u>	<u>ID<sub>50</sub> (μM)</u>	<u>Potency relative to Idoxuridine</u>
Vidarabine	16	6
Idoxuridine	1	100
Acyclovir	0.1	1000

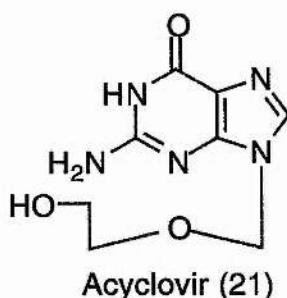
The selectivity and low cytotoxicity may be attributed to the specificity of acyclovir towards virally-induced enzymes over host cell enzymes. Acyclovir is converted into its monophosphate by virally specified thymidine kinase 30-120 times faster than by host cell thymidine kinase and subsequently to di- and triphosphates by cellular enzymes. Incorporation into the growing DNA chain by HSV DNA polymerase thus allows competitive inhibition of the natural nucleosides<sup>31</sup> at the active site and action as a chain terminator once incorporated<sup>32</sup> due to the absence of the lower sugar portion.

Since its inception, acyclovir has been a market leader and the benchmark by which all competitors are measured. First marketed as an injectible solution in 1982 (for the treatment of HSV2) and later accepted by the FDA in oral dosage form, it is now available over the counter as a topical treatment for recurrent cold sores (HSV1) and is regularly



prescribed for the treatment of shingles (VZV).

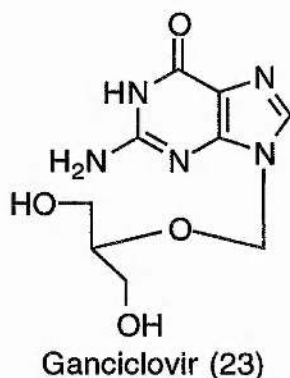
Despite being an excellent therapy, acyclovir has very poor bioavailability. Attempts to improve GI (gastrointestinal) absorption by oral administration of a prodrug, namely 6-deoxyacyclovir<sup>33</sup> (Desciclovir), met with considerable success *in vivo*, oxidation occurring via xanthine oxidase to the active acyclovir. However further work has been limited and furthermore the low bioavailability has been proposed to be a factor in the low cytotoxicity.



## 1.6 Commercial competitors of acyclovir

Although acyclovir has been extensively pursued for its clinical efficacy, it has also been responsible for increased activity in the search for selective antiviral nucleoside analogues in what is an expanding and very profitable market and has lead to competitor companies discovering among others ganciclovir (Syntex/Merck), 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine, DHPG, 2'-nor-2'-deoxyguanosine, 2'NDG, BIOLF-62 or BW759U (23), which more closely resembles the natural deoxynucleoside and whose activity has been reported independently by several groups<sup>34</sup>. Like acyclovir, ganciclovir (23) is selective for the virally infected cells yielding the monophosphate which is further phosphorylated by host cell kinases and accepted as a substrate by the viral DNA polymerase affording another level of selectivity over the host. Ganciclovir is a broadly active antiherpetic agent which is substantially devoid of toxicity, more water soluble and bioavailable than acyclovir but is similarly active against HSV1, HSV2 and VZV. In addition, ganciclovir is more potent against HCMV and Epstein Barr virus well below cytotoxic levels, is superior *in vivo*<sup>35</sup> and is active against acyclovir resistant strains of

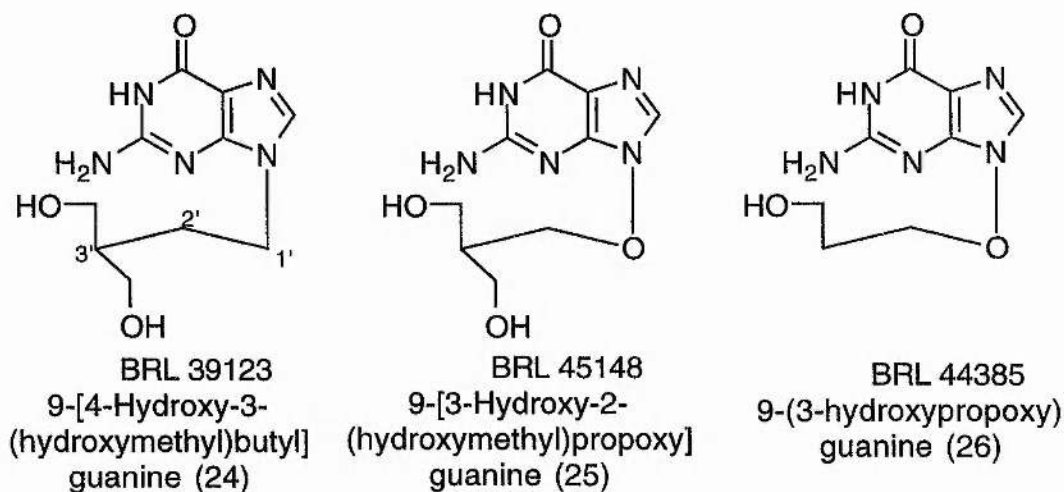
HSV1<sup>36</sup> which do not possess a virally specified thymidine kinase but have a DNA polymerase suggesting a different mode of action<sup>34</sup>. The activity against HCMV bears great interest for the treatment of severe cytomegalovirus infections (including pneumonia, chorioretinitis and organ damage) in immunosuppressed (recipients of organ and bone marrow transplants) and immunocompromised (AIDS) patients. Although there is no HCMV encoded thymidine kinase sufficient selectivity is presumably attained at the virally encoded DNA polymerase level<sup>37</sup> (phosphorylation still occurring to a greater degree in infected cells).



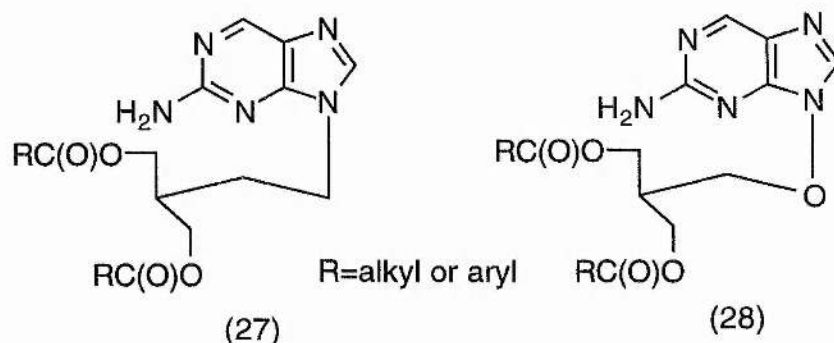
However, ganciclovir has been cited as having adverse effects (bone marrow toxicity) in humans and animals<sup>38</sup>. In bone marrow transplant patients, maintenance therapy is required until the immune system has recovered sufficiently whereas in AIDS patients, indefinite maintenance may be required to prevent disease progression as the latent infection is not eradicated. This results in adverse haematological effects which although reversible, may require the cessation of therapy<sup>39</sup> and hence the risk of further opportunistic infection.

In parallel with the research being carried out to produce acyclovir and ganciclovir, a group at SmithKline Beecham was looking at the potential of an anti-herpes therapy in the carba-analogue of ganciclovir, BRL39123 or 9-[4-hydroxy-3-(hydroxymethyl)butyl] guanine (24). This was first reported in 1972<sup>40</sup> and had notable antiviral properties<sup>41</sup>. BRL39123 had similar *in vitro* activity against HSV1 and HSV2 but like acyclovir was

inactive with mutant HSV strains encoding no thymidine kinase: polyphosphorylated metabolites were proposed to be competitive inhibitors of deoxyguanosine triphosphate at the viral DNA polymerase level<sup>42</sup> indicating an acyclovir-like mode of action and chain termination of the growing DNA chain.



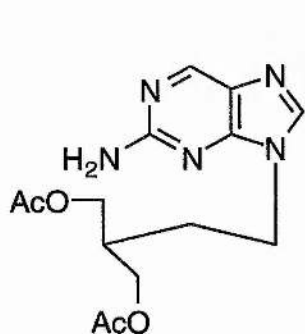
Later work by the same group produced some interesting analogues with good activity. In BRL45148 (25) the  $-\text{CH}_2-$  at the 1' position of the acyclic moiety had been replaced by an oxygen atom to give an N-O linkage to the guanine base and in BRL44385 (26) one of the 3' hydroxymethyl groups has also been removed. Little had been reported on the stability of these N-O bonds; however, studies have proved them to be stable under a variety of conditions<sup>43</sup>. Poor bioavailability was also a problem here and prodrugs in the form of 6-deoxy and ester congeners (27) and (28) were synthesised and investigated for GI absorption and conversion into the active drug. These have provided in varying degrees, substantially higher concentrations of the active compound in the blood than are obtainable by direct oral administration of the active agent itself<sup>43,44</sup>.



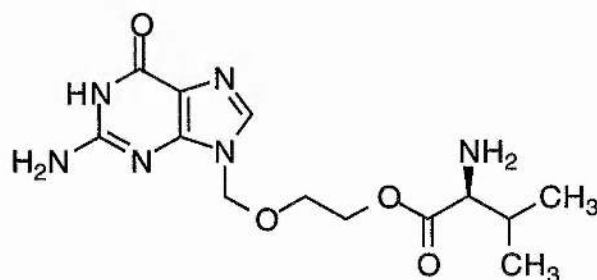
**Table 1.2 Comparison of the in vitro activity of Acyclovir and SB compounds<sup>42, 43</sup>**

<u>Compound</u>	<u>IC<sub>50</sub> (μM)</u>		
	<u>HSV1</u>	<u>HSV2</u>	<u>VZV</u>
Acyclovir	6.7	2.5	20
BRL 39123	6.4	6.4	—
BRL 45148	2.1	0.71	4.4
BRL 44385	5.9	5.9	11

Many other modifications to the base<sup>45</sup> and acyclic side chain<sup>46</sup> at positions C-1', C-2' and C-3' have also been investigated producing a comprehensive structure-activity relationship of these acyclonucleosides. This research has resulted in the introduction of the new antiviral drug, Famvir by Smith-Kline Beecham. Around the same time Glaxo-Wellcome received FDA approval for and introduced its successor to acyclovir, Valacyclovir (the patent of acyclovir having almost expired) as a treatment for HSV and herpes zoster (shingles). Valacyclovir (Valtrex, 30) is the L-valyl ester prodrug of acyclovir providing improved bioavailability via involvement of a stereospecific transport process and hence requiring a less frequent dosing schedule<sup>47</sup>.



Famvir (29)  
SmithKline Beecham

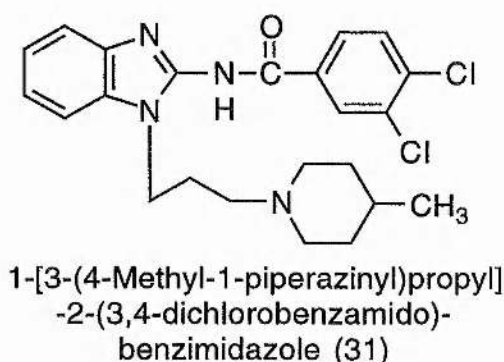


Valacyclovir (30)  
(Valtrex)

## 1.7 Benzimidazoles and benzimidazole *N*-oxides of biological interest

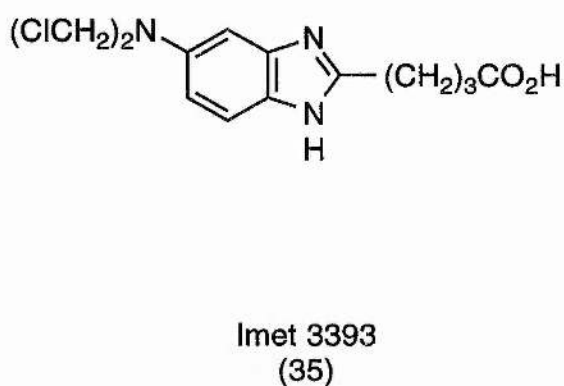
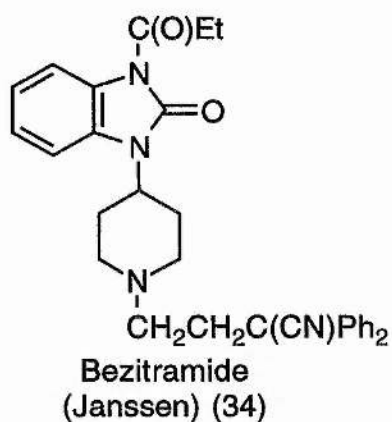
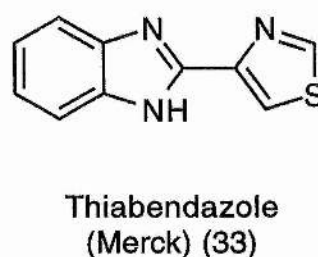
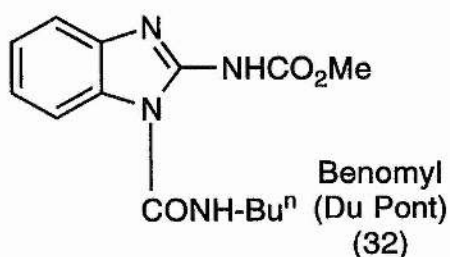
Rigorous research into benzimidazoles and benzimidazole *N*-oxides has also been undertaken during this explosion of intensive nucleoside research producing an array of compounds with interesting biological activities. This was stimulated, in part at least, by the discovery of a benzimidazole riboside, 5,6-dimethyl-1-( $\alpha$ -D-ribofuranosyl) benzimidazole, as an integral part of the structure of vitamin B<sub>12</sub> through the elucidation of its structure by X-ray crystallography<sup>48</sup>. Vitamin B<sub>12</sub> contains a highly substituted and reduced corrin ring and an unusual nucleoside monophosphate which has an  $\alpha$ -glycosidic bond (in contrast to the  $\beta$ -glycosidic bond of the nucleic acids, page 2). The discovery of a benzimidazole as part of a natural biological system, if taken together with the structural similarities benzimidazoles bear to the natural purines, may be responsible for the increase in interest in the chemistry associated with this area.

A certain amount of commercial success has arisen from biologically active benzimidazoles such as compound (31), a non-steroidal anti-inflammatory for the treatment of chronic inflammatory disease<sup>49</sup>.



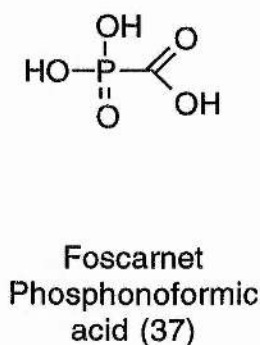
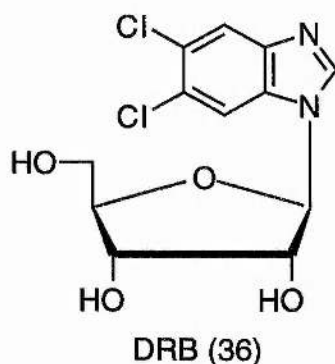
Although many other similar compounds have been developed, the most important commercial benzimidazole is the fungicide Benomyl<sup>50</sup> (methyl 1-(butylcarbamoyl) benzimidazol-2-ylcarbamate, 32). It was introduced in 1967 by Du Pont and used in the treatment of a wide range of fungi and mites but its use has recently declined owing to the development of resistance in pathogenic fungi. Other such active agents include the

anthelmintic and fungicide Thiabendazole (33)<sup>50</sup>, the analgesic Bezitramide (34)<sup>50</sup> and the anticancer agent Imet 3393 (35)<sup>50</sup>.



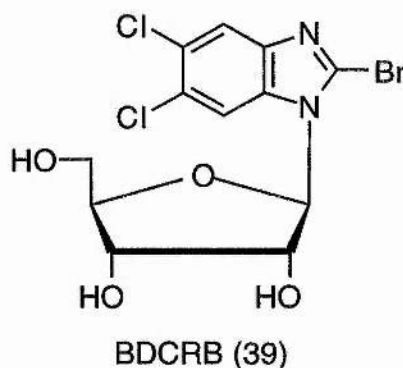
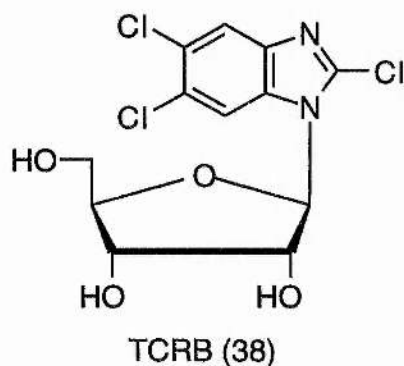
### 1.8 Benzimidazole ribonucleosides

More importantly, in the 1950s, Tamm and co-workers reported the activity of a benzimidazole riboside [(5,6-dichloro-1-β-D-ribofuranosyl)benzimidazole, DRB (36)] against a number of RNA and DNA viruses. However the antiviral activity was poorly separated from cytotoxicity, affecting multiple cellular processes and hence having no utility as an antiviral agent<sup>51</sup> but with possible implications as an anticancer agent.



In an effort to find possible anticancer agents modifications to both the benzene ring and the sugar moiety of DRB were investigated by Townsend, *et al.*<sup>52</sup>, in the 1960s. These modifications resulted in a decrease in anticancer activity relative to DRB (36) itself. However introduction of a halogen at the 2-position produced compounds which although of little anticancer activity possessed low cytotoxicity and surprisingly were found to inhibit HCMV replication.

These trihalogenobenzimidazole ribonucleosides, TCRB (38) and BDCRB (39) possess good activity against HCMV at a low cytotoxicity and act by a unique mechanism in the inhibition of the DNA processing<sup>53</sup>. Current therapies for HCMV, including ganciclovir (previously mentioned) and foscarnet (37), suffer from the twin drawbacks of low potency and significant adverse side effects. Foscarnet<sup>54</sup> resembles pyrophosphate and occupies its position in the nucleotide binding site of DNA polymerase to prevent its binding. It is active against all herpesvirus DNA polymerases and HIV RT but has side effects associated with the chelation of divalent metal ions such as calcium.

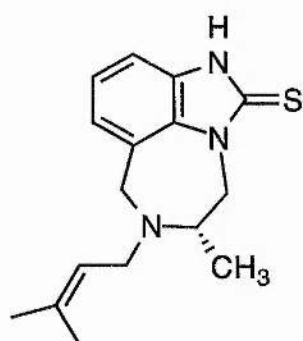


Investigation of further substituents at the 2 position and on the benzene ring have provided no better activity than that of TCRB and BDCRB<sup>51,53,54</sup> (see table overleaf).

**Table 1.3 Activity and toxicity of benzimidazole ribosides and established treatments against human cytomegalovirus<sup>51,53</sup>**

Compound	IC <sub>50</sub> (μM)	Toxicity (μM)
DRB	42	24
TCRB	2.9	238
BDCRB	0.7	118
DHPG	7.4	>100
acyclovir	86	>100
foscarnet	39	>100

Other work by Townsend *et al.* has focussed on the activity of benzimidazoles as antifilarials<sup>55</sup>, antineoplastics and anti-HIV agents<sup>56</sup>. The latter, non-nucleosidic inhibitors of reverse transcriptase, targetting an allosteric site are only active against HIV1 (not HIV2) and are analogues of the thoroughly investigated TIBO<sup>57</sup> tricyclic system (40) but with a scission of the diazepine ring to give 2-mercaptobenzimidazole derivatives (41).



TIBO Analogue  
(R82150) (40)

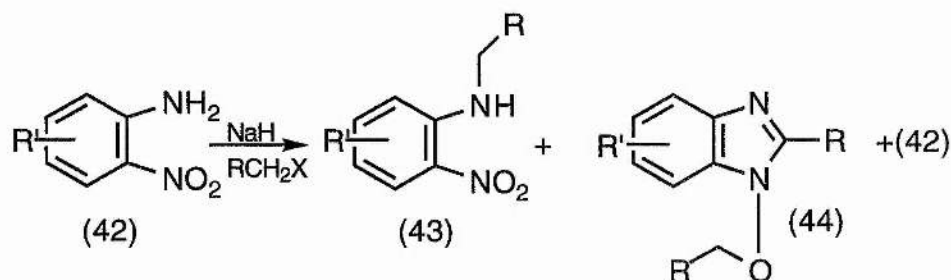


R<sub>1</sub>=H, CH<sub>3</sub>  
R<sub>2</sub>=alkyl, aryl  
(41)

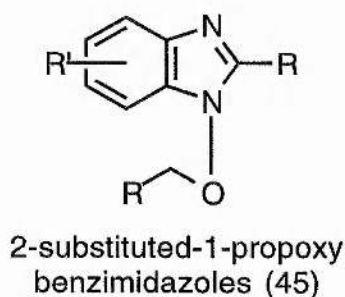
In an effort to produce compounds similar to the TIBO analogues (40) and those prepared by Townsend *et al.* (41), Gardiner and co-workers at UMIST<sup>58</sup> discovered a



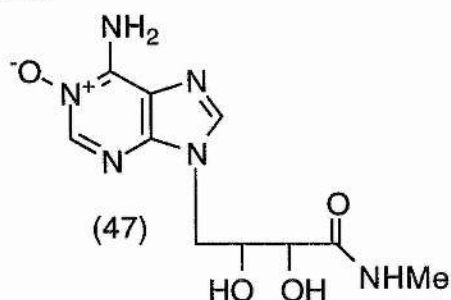
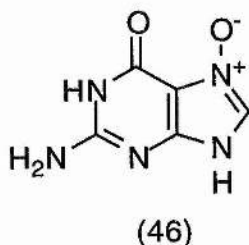
cascade of reactions which produced 2-substituted benzimidazole *N*-oxides which are also alkylated on the oxygen without further reaction (44). Alkylation of substituted *o*-nitroanilines (42) with alkyl, aryl and allyl halides using sodium hydride as the base was shown not to stop at either the *N*-substituted-*o*-nitroaniline (43) or the subsequent 2-substituted benzimidazole *N*-oxide (see page 22) but proceed directly to the *O*-substituted benzimidazole to furnish a final structure (44) and return only a small amount of unreacted starting material.



When alkyl halides are reacted with *o*-nitroanilines possessing a variety of substituents on the benzene ring, the *N*-alkoxyheteroaromatic compounds produced (45) have similar characteristics to many active biological species and were found to possess moderate activity ( $\mu\text{M}$  range) against HIV RT<sup>59</sup>. Similar products may be formally derived via the alkylation of functionalised benzimidazole *N*-oxides.



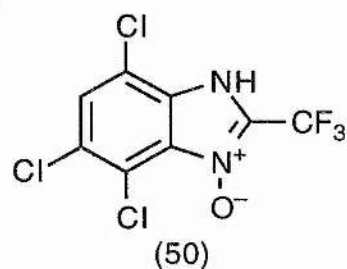
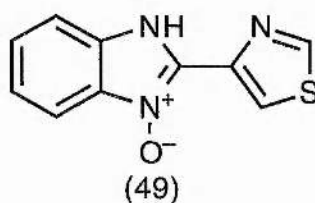
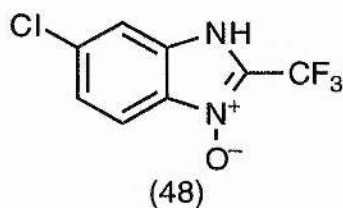
Reference has previously been made to the biological activity of 9-alkoxypurines (see page 13); some purine *N*-oxides have biological activity in their own right. The

Purine *N*-oxides

*N*-oxides of both guanine and a substituted adenine have been found to possess biological properties: the 7-oxide of guanine (46) has been reported to have antimicrobial, antiviral and antitumour activity<sup>60</sup> and the 1-oxide of the adenine (47) has been reported as a hypocholesteremic<sup>61</sup>.

1.9 Benzimidazole *N*-oxides

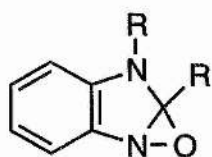
Several benzimidazole *N*-oxides have also been found to have a variety of commercially exploitable biological actions including herbicides (48)<sup>62</sup>, nematocides (49)<sup>63</sup> and anthelmintics (50)<sup>64</sup>.

*N*-oxides with biological activity

The correct structure for the benzimidazole *N*-oxide (52) was only assigned in 1951<sup>65</sup>, previously the oxbenzimidazoles (as they were called) had been assigned a tricyclic structure (51). They were first discovered, apparently independently, by both von Niementowski in 1887<sup>66</sup> and Bankiewicz in 1888<sup>67</sup>. After discovery, their chemistry suffered almost complete neglect until the early 1950s when heterocyclic *N*-oxides became

a very active area of research.

Unlike other heterocyclic *N*-oxides the benzimidazoles cannot be oxidised to the *N*-oxide either by direct<sup>68</sup> or indirect<sup>69</sup> methods and their syntheses are dominated by routes leading to the final formation of the C(2)-N(3) bond in a cyclisation reaction. However, they can be reduced, like other heterocyclic *N*-oxides, by a variety of conditions to the parent heterocycle.



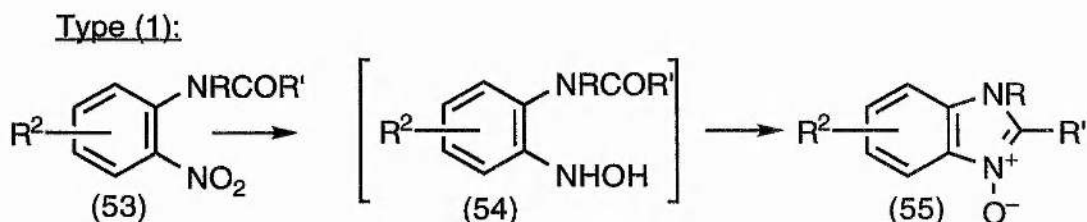
Oxbenzimidazole (51)



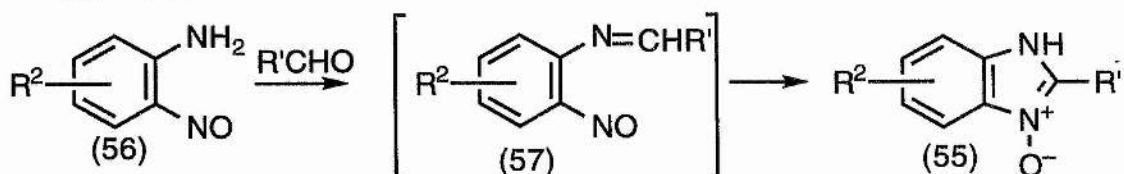
Benzimidazole *N*-oxide (52)

The cyclisations fall into three basic types (1,2,3 below):

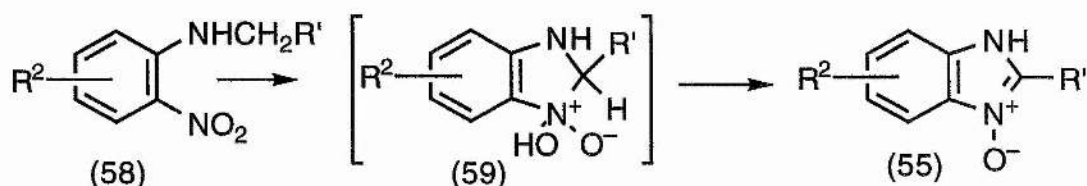
Type 1 involves the condensation of a hydroxylamine (54), resulting from the partial reduction of an *o*-nitroanilide (53), with an acidic carbonyl. Initially the *N*-oxides were by-products of the total reduction of the *o*-nitroanilide but careful manipulation of the reductive conditions<sup>70</sup> can lead to significant yields of the benzimidazole *N*-oxides (55).



The second type of cyclisation involves the interaction of an azomethine carbon with an *o*-nitroso group (57); the simplest of this type of reaction being the acid catalysed condensation of an *o*-nitrosoaniline (56) with an aldehyde (benzaldehyde)<sup>71</sup>; however the *o*-nitrosoanilines are very difficult to prepare and are themselves an unstable species.

Type (2):

Finally, *o*-substituted nitroarenes (58) may be cyclised to benzimidazole *N*-oxides (type 3, 55) with the condensation of the nitro group and a nucleophilic centre in the *ortho* substituent (59)<sup>72</sup>. The *ortho* substituent must be alkyl- or aryl-amino, and the reactive centre activated by an electron accepting group such as nitrile, ester, ketone or amide resulting in a 2-substituent in the final product. These cyclisations may be acid, base or thermally induced. With the aforementioned activating groups the cyclisation occurs under extremely mild conditions; weaker electron acceptors (e.g. R'=aryl in the scheme below) require more extreme conditions.

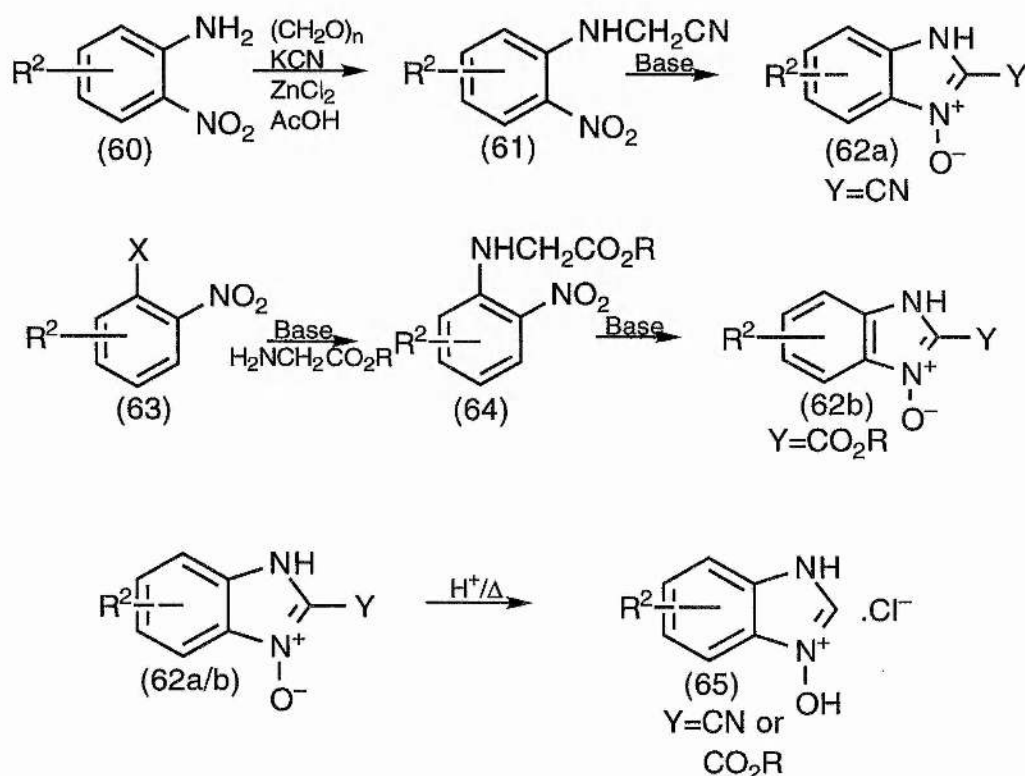
Type (3):**1.10 A general synthetic route to benzimidazole *N*-oxides**

During the past 20 years, this research group in St. Andrews has been investigating the synthesis of benzimidazole *N*-oxides and more recently working towards the production of a general synthetic route for the introduction of functionality to the carbocyclic ring; thus enabling the synthesis of benzimidazole *N*-oxides which are structurally similar to the natural purines, adenine and guanine.

The reported<sup>73</sup> synthetic route is based upon the observation<sup>74</sup> that benzimidazole-2-carboxylic acid *N*-oxides undergo facile decarboxylation when heated in solvents even at

relatively low temperatures ( $\sim 80^\circ\text{C}$ ). Cyanomethylation<sup>75</sup> of a suitable *o*-nitroaniline (60) and subsequent cyclisation affords the 2-cyanobenzimidazole 3-oxide (62a). Hydrolysis in concentrated hydrochloric acid yields the hydrochloride salt of the *N*-oxide (65), formally derived through the hydrolysis of the nitrile to the carboxylic acid and spontaneous decarboxylation upon heating. The free *N*-oxide is obtained by a simple work-up procedure involving aqueous ammonia<sup>73</sup>. The cyanomethylation step is simply modified by varying the quantity of Lewis acid according to the basicity/nucleophilicity of the amine: the less basic/nucleophilic amines requiring a greater amount of Lewis acid.

In certain cases *N*-*o*-nitrophenylglycine esters (64) may conveniently replace the *N*-cyanomethyl-*o*-nitroanilines (61). The reaction of a halogenonitrobenzene (63) with a glycine alkyl ester affords a nitrophenylglycine ester (64) which will cyclise like the corresponding nitriles under mild, basic conditions to produce a benzimidazole *N*-oxide carrying an ester group at C-2 (62b). This method removes the need to use potassium cyanide, but is applicable only when the starting halogeno compound is available.



1-Unsubstituted benzimidazole 3-oxides are tautomeric with *N*-hydroxybenzimidazoles, the position of the equilibrium having been studied extensively for certain derivatives. In these cases the *N*-oxide predominates in aqueous solution. In other solvent systems as the hydrogen bonding power (polarity) of the solvent decreases, so the proportion of *N*-hydroxy tautomer increases<sup>76</sup>. In the remainder of this thesis, however, these compounds are all referred to, for convenience, as benzimidazole 3-oxides.



Benzimidazole 3-oxides are weakly basic<sup>76</sup> and the 1-unsubstituted benzimidazole 3-oxides are also weakly acidic, requiring only mild bases to effect deprotonation. Alkylation of the *N*-oxide species (in the majority of cases studied so far) in the presence of a base and an alkyl halide leads to the formation of 1-alkoxybenzimidazoles. The benzimidazole is thus attached to a side chain<sup>77,78,79</sup> via a N-O-C linkage.

### 1.11 Aims

The aim of this research is to exploit the already established general synthetic route to benzimidazole 3-oxides to produce novel guanine analogues in which an acyclovir-like appendage is attached to the base via alkylation of the *N*-oxides on the oxygen atom.

Initial routes will investigate the production of a 5-nitrobenzimidazole 3-oxide with the express purpose of later reduction to furnish the amino group and consequent guanine analogue while leaving the N-O-C linkage intact. Possibilities for expansion of the synthetic route to produce nucleoside analogues possessing functionality at C-2, as proposed to be required for the maintenance of activity in the Townsend series of benzimidazole ribonucleosides will be explored as well as the production of similar

benzimidazole ribonucleosides will be explored as well as the production of similar polyhalogenated nucleoside analogues.

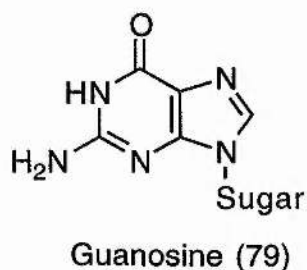
The biological target is initially postulated to be the herpes group of viruses for which HSV1 testing will be carried out in-house. More importantly, HCMV is also a target and as has been previously outlined, a potent, effective and non-toxic therapy for the treatment of its manifestations in the immunosuppressed/compromised has not yet been discovered. Testing for such activity and against a more expansive panel of viruses will be undertaken outwith the university.

## Chapter 2

### *N*-Alkoxybenzimidazoles related to guanosine

#### 2.1 Introduction

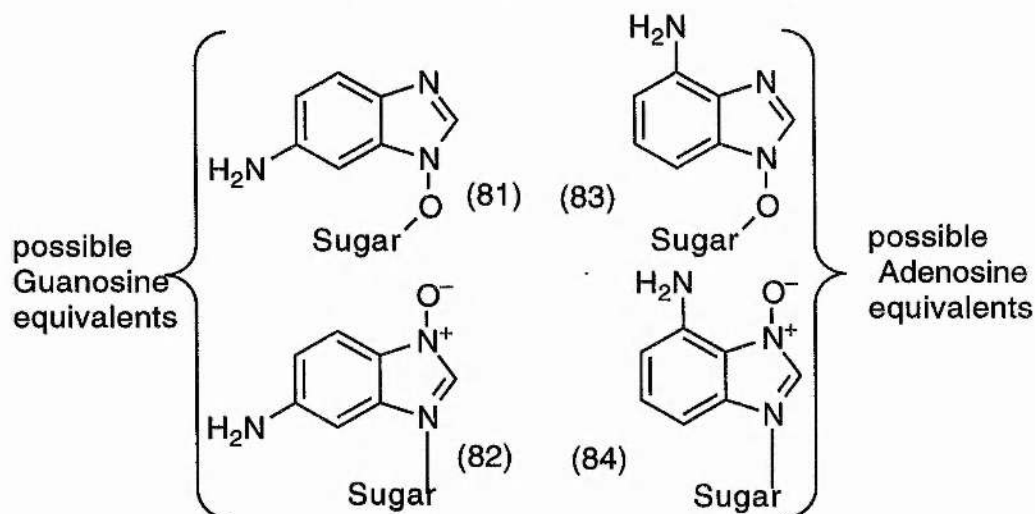
This research group in St. Andrews has provided, over the years, a general synthetic route to benzimidazole *N*-oxides with a variety of substituents on the carbocyclic ring; these benzimidazole oxides are unsubstituted at the other nitrogen and may also be unsubstituted at C-2. Benzimidazole *N*-oxides may be synthesised with functionality similar to the natural purines; compounds with substituents such as 5- and 6-amino groups could be regarded as guanosine analogues [cf. (79), (81) and (82)] and those with the 4- or 7-amino substituent as adenosine analogues [cf. (80), (83) and (84)]. Benzimidazole *N*-oxides with amino functionality on the benzene ring may be synthesised through the reduction of a nitrobenzimidazole *N*-oxide. A suitable point in the synthesis of the benzimidazole oxide would need to be chosen for the reductive step. Alternatively, the amino groups may be protected at the beginning of the synthetic route, the exocyclic nitrogen at the correct oxidation level, and deprotected after the formation of the required benzimidazole *N*-oxide. Reaction of the benzimidazole *N*-oxide with a suitable acyclic halide or sugar derivative may then lead to nucleoside analogues in which the attachment to the benzimidazole (base) is via an N-C (natural) or an N-O-C (unnatural) linkage; both are theoretically possible.



The excellent activity of market-leading antiviral compounds such as acyclovir (21) and famvir (29), coupled with their structural similarity to the natural nucleosides makes the



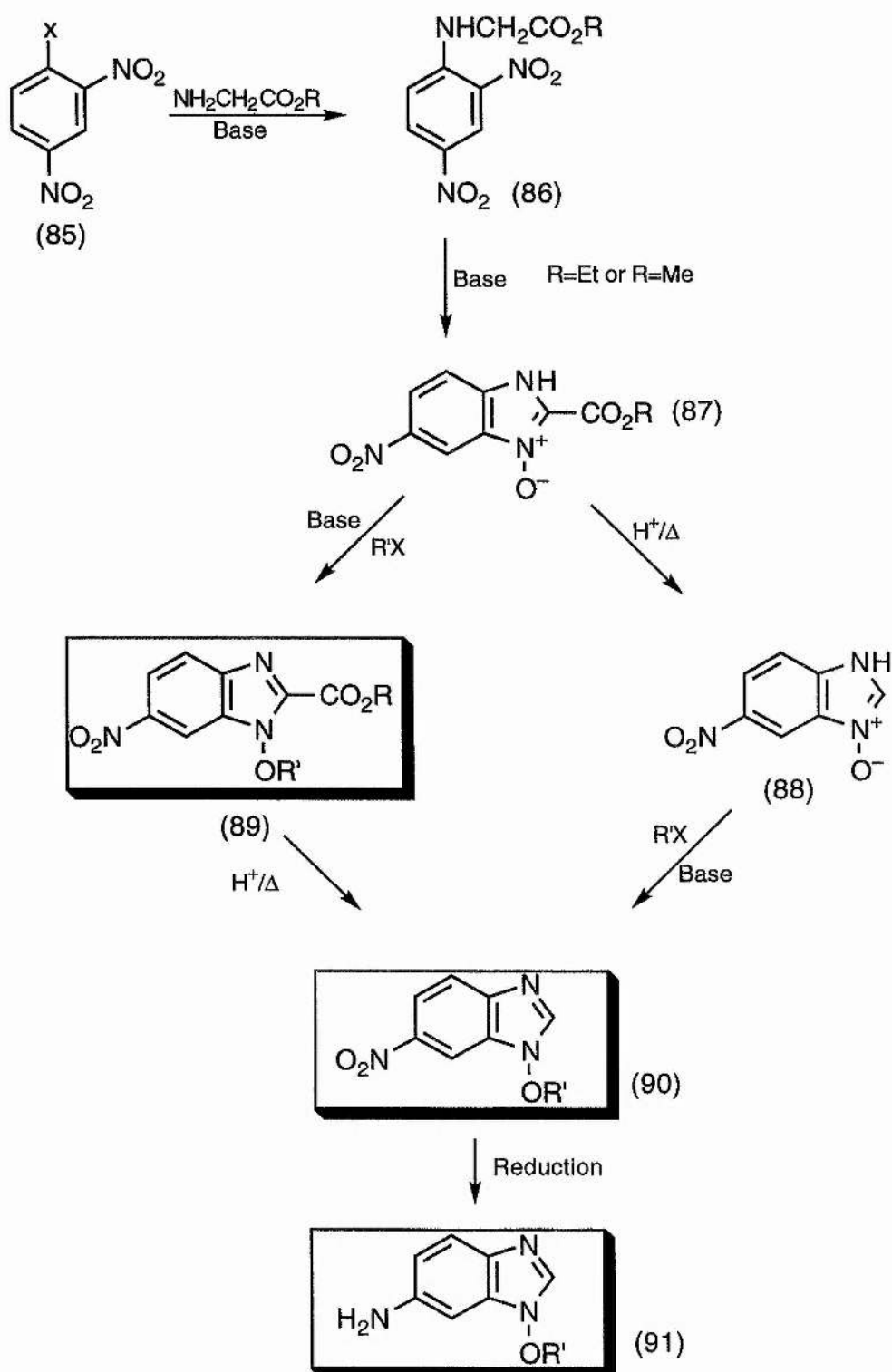
study of benzimidazole nucleoside analogues particularly important due to their structural similarities to both the natural substrates and the commercially successful antiviral agents.



The most straightforward synthetic route leading to benzimidazole *N*-oxides is via the cyclisation of *N*-(activated alkyl)-*o*-nitroanilines with the subsequent removal of the functionality at C-2, if desired, by hydrolysis/decarboxylation in concentrated mineral acid.

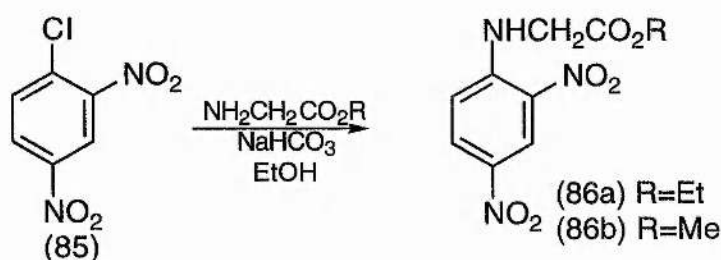
The initial aim was to find a route to a guanosine analogue. It was decided, in view of previous work<sup>73</sup>, to proceed via a nitrobenzimidazole oxide and reduce the nitro group to the required amino functionality in the latter stages of the synthesis. The proposed route would involve reaction of a halogenonitrobenzene (85) and a glycine alkyl ester (ethyl or methyl) with subsequent cyclisation of the nitrophenylglycine ester (86) to the benzimidazole *N*-oxide with the ester functionality at C-2 (87). A choice of routes in the synthesis was then available; either alkylation or hydroxyalkylation first, giving (89), followed by the hydrolysis/decarboxylation, or the alternative process of hydrolysis/decarboxylation to (88) prior to (hydroxy)alkylation giving (90). The choice of two routes could prove to be a valuable tool in the synthetic route by providing more flexibility. Little was known prior to this investigation concerning the stability of the N-O-C linkage (89)<sup>78,79</sup> under the hydrolytic conditions required to remove the functionality from C-2, or indeed under any reaction conditions (see scheme 2.1 overleaf: boxed structures (89), (90) and (91) are of potential biological interest).

**Scheme 2.1**  
**Proposed synthetic route**

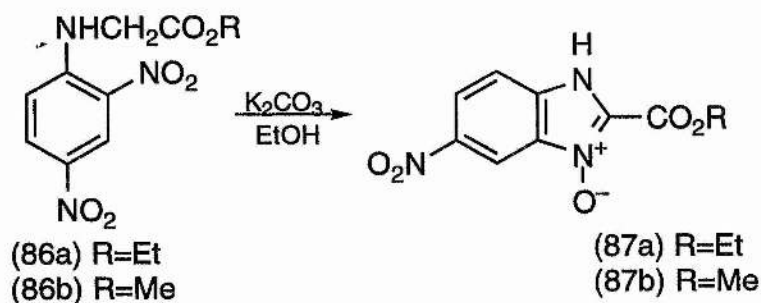


## 2.2 Synthesis of 5-nitrobenzimidazole 3-oxide

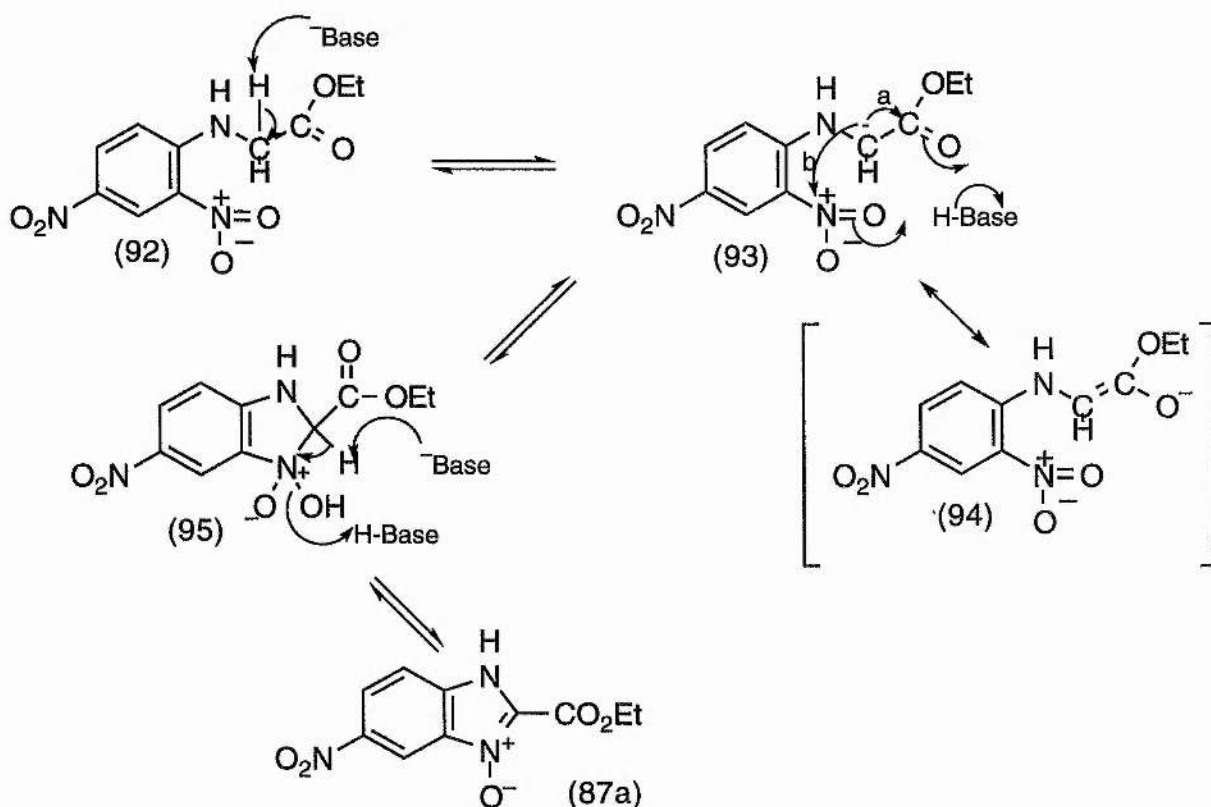
As stated in the literature<sup>73</sup>, 2,4-dinitrophenylglycine ethyl (86a) and methyl (86b) esters were synthesised from 1-chloro-2,4-dinitrobenzene (85) and the relevant glycine alkyl ester hydrochlorides. Only mild conditions are required due to the electrophilicity of the carbon atom which is both *ortho* and *para* to the nitro groups. [In the case of ring substituents which are electron-donating, either a more labile halogen atom (fluorine) or a more nucleophilic nitrogen atom, or both, are required. ]



The cyclisation step was reported<sup>73</sup> to be effected under extremely mild conditions (piperidine in ethanol): however, this procedure could not be repeated. Numerous attempts involving the varying of reaction times and the use of freshly purified and different batches of piperidine, still only yielded trace amounts of product and returned much of the starting material unchanged. When anhydrous potassium carbonate was used in place of piperidine, the desired product (87a/b) was obtained in a cleaner reaction and with a reasonable yield; both the ethyl (86a) and methyl (86b) esters cyclised equally well. It is suggested that the piperidine used by previous workers was of poor quality and contained sufficient water that its attempted drying over potassium hydroxide resulted in the partial dissolution of some of the hydroxide, which in turn had acted as the base in the previous cyclisations.

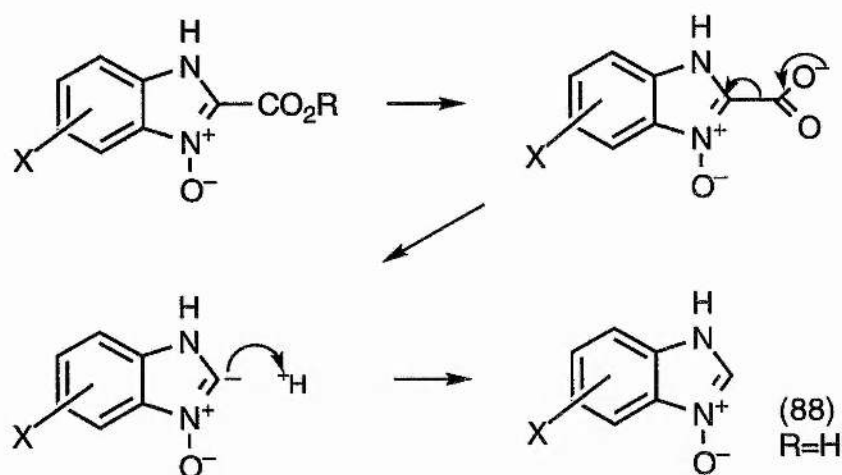


The cyclisation is most simply formulated by the base (potassium carbonate) abstracting a proton from the active methylene group of (92). The intermediate carbanion (93) (stabilised by the ester group) attacks at the electrophilic centre of the nitro group in an intramolecular aldol-type reaction giving (95), after which a molecule of water is eliminated to furnish the *N*-oxide (87a) with the ester functionality at C-2.



Having synthesised both the ethyl (87a) and methyl (87b) 5-nitrobenzimidazole-2-carboxylate 3-oxides, a possible branch point in the synthesis had been reached. The next

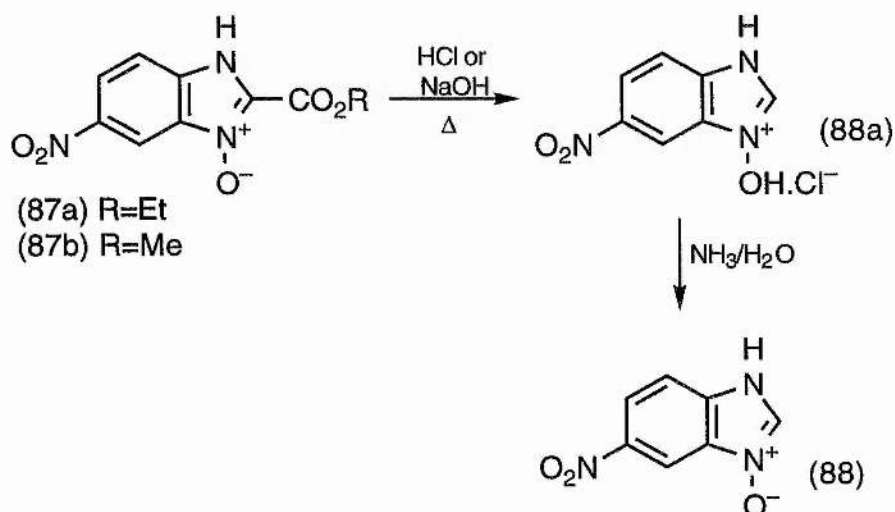
step could either be alkylation on the oxygen atom (N-1 was also theoretically possible) or hydrolysis/decarboxylation of the ester (see scheme 2.1, page 28). Hydrolysis/decarboxylation will be dealt with in the first instance. It has been reported<sup>74</sup> that benzimidazole-2-carboxylic acids (96) undergo facile decarboxylation when heated in solvent at moderate temperatures to yield the benzimidazole oxide unfunctionalised at C-2. This had provided the ideology for the removal of either nitrile or ester under the perfected hydrolytic conditions.



Heating of the alkyl 5-nitrobenzimidazole-2-carboxylate 3-oxides (87a) or (87b) in concentrated hydrochloric acid yields the *N*-oxide (88a) as its hydrochloride salt which crystallises out of the reaction mixture upon cooling. Such hydrochloride salts have characteristic <sup>1</sup>H nmr spectra in which H-2 has a shift of approximately δ 9.5-10. The free *N*-oxide (88) is reached through a simple work-up in aqueous ammonia, the product crystallising out of solution on partial concentration under reduced pressure.

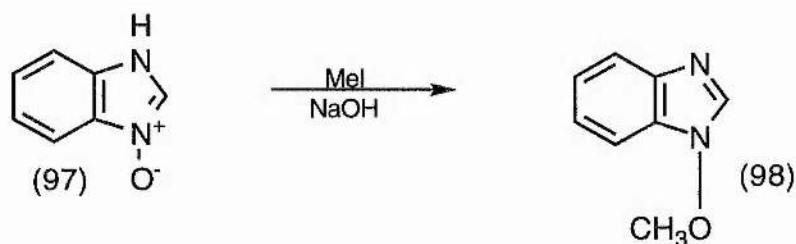
The hydrolysis/decarboxylation reactions of (87a/b) to produce the *N*-oxide (88) were also attempted in a basic medium in order to introduce further flexibility to the synthetic route. The esters were stirred in an aqueous sodium hydroxide medium for 2 hours and the precipitates collected. In the case of the ethyl ester (87a) the product was isolated in good yield but in the case of the methyl ester (87b), a mixture of the *N*-oxide

(88) and its hydrochloride salt (88a) was isolated. The reaction was pursued no further in view of the success of the acidic hydrolysis route.



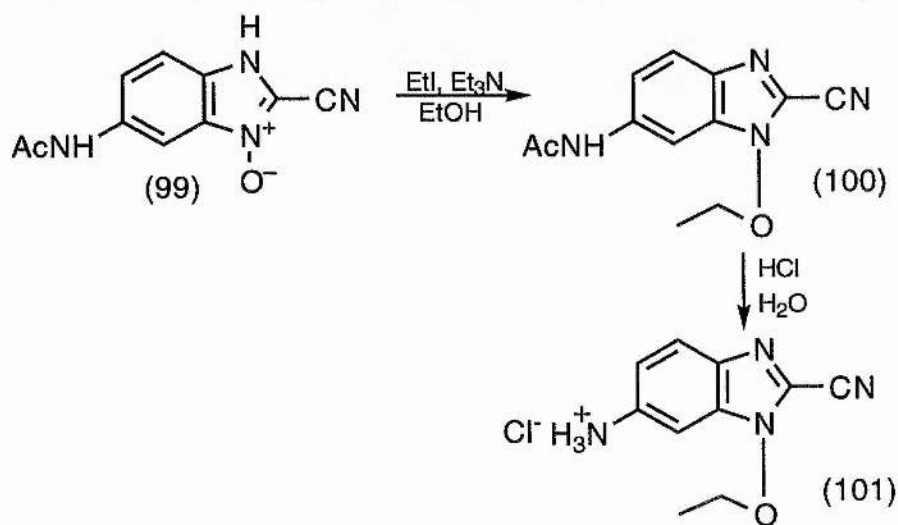
### 2.3 *O*-Alkylation of the benzimidazole oxide

The 5-nitrobenzimidazole 3-oxide (88) was then available via acidic or basic hydrolysis for the attempted alkylation upon the oxygen atom to provide the desired N-O-C linkage. Benzimidazole *N*-oxides (97) were known<sup>76,81</sup> to undergo *O*- rather than *N*-alkylation with an alkyl halide in the presence of a base to give, e.g. (98).



Initial attempts at alkylation were centred upon a simple model system using ethyl iodide. This had been used in earlier work by Moody<sup>81</sup> to afford 1-ethoxy-2-cyano-6-acetamidobenzimidazole (100) from the corresponding *N*-oxide (99) using triethylamine as the base. Removal of the acetyl protective group gives the amine (101) as its hydrochloride

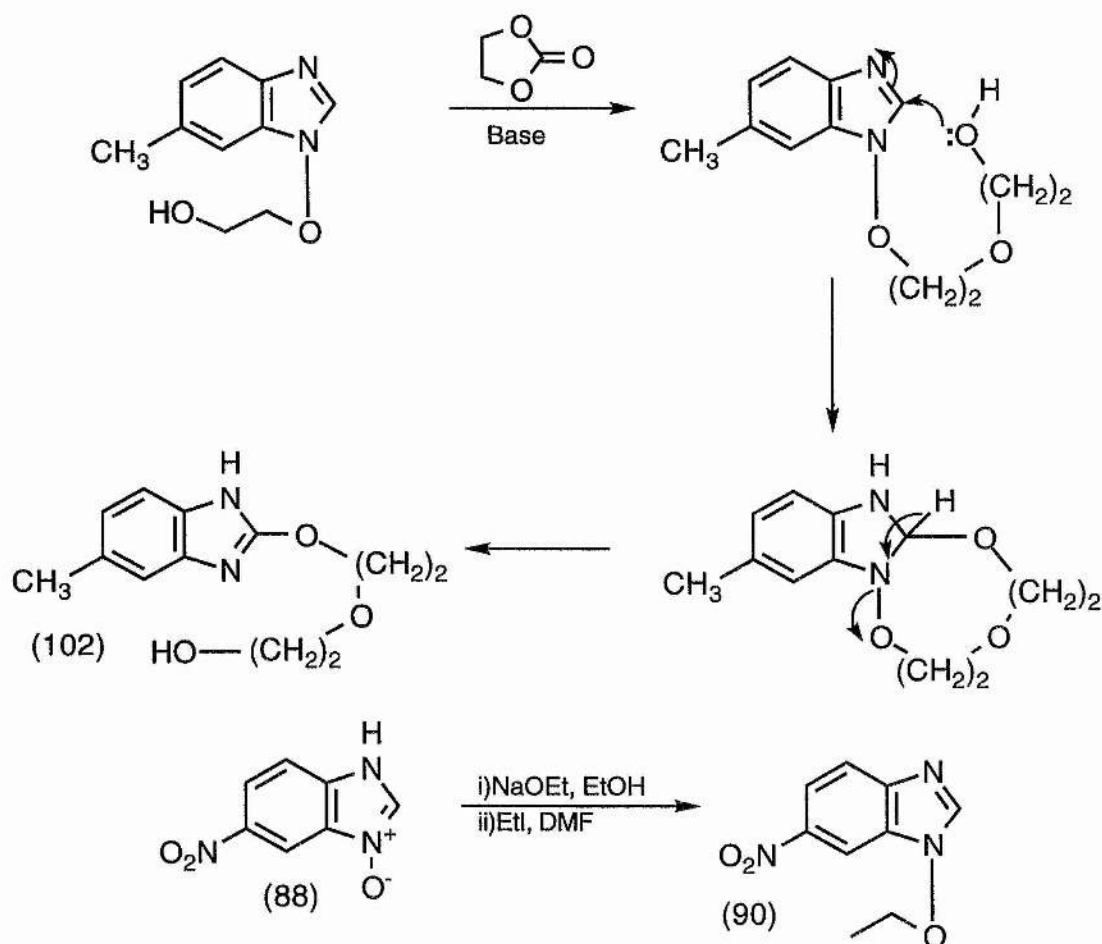
with the nitrile still intact. Attempts by Moody to react both benzimidazole *N*-oxide and ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide with  $\alpha$ -acetobromoglucose (2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide) proved partially successful, in that the products were detected spectroscopically but proved impossible to isolate in a pure form.



The use of more complex acyclic alkylating agents by McFarlane<sup>82</sup> had also produced little success. Attempted reaction of 5-methylbenzimidazole 3-oxide with both 2-bromoethanol and 3-bromopropan-1-ol returned only unreacted starting material. Limited success was met in the reaction with this benzimidazole oxide with ethylene carbonate, but on attempted purification a resinous material was produced which had lost the H-2 resonance in the  $^1\text{H}$  nmr. A molecular ion of  $M^+ = 236$  in the mass spectrum led to the proposal of structure (102) for the product through the mechanism shown overleaf, unfavourable side reactions having resulted in the loss of the desired N-O-C linkage and the attachment of the side chain at C-2. Failure was also met in attempts at the reaction with  $\alpha$ -acetobromoglucose the product proving impossible to isolate in pure form.

Following literature methods<sup>81,82</sup>, the *N*-oxide (88) was dissolved in DMF and addition of a solution of sodium ethoxide followed by ethyl iodide furnished 1-ethoxy-6-nitrobenzimidazole (90) but in low yield. [Ethanol was initially used as the solvent but precipitation occurred on addition of the base and the reaction proceeded no further. The isolated product seemed to be the sodium salt of unchanged starting material which had

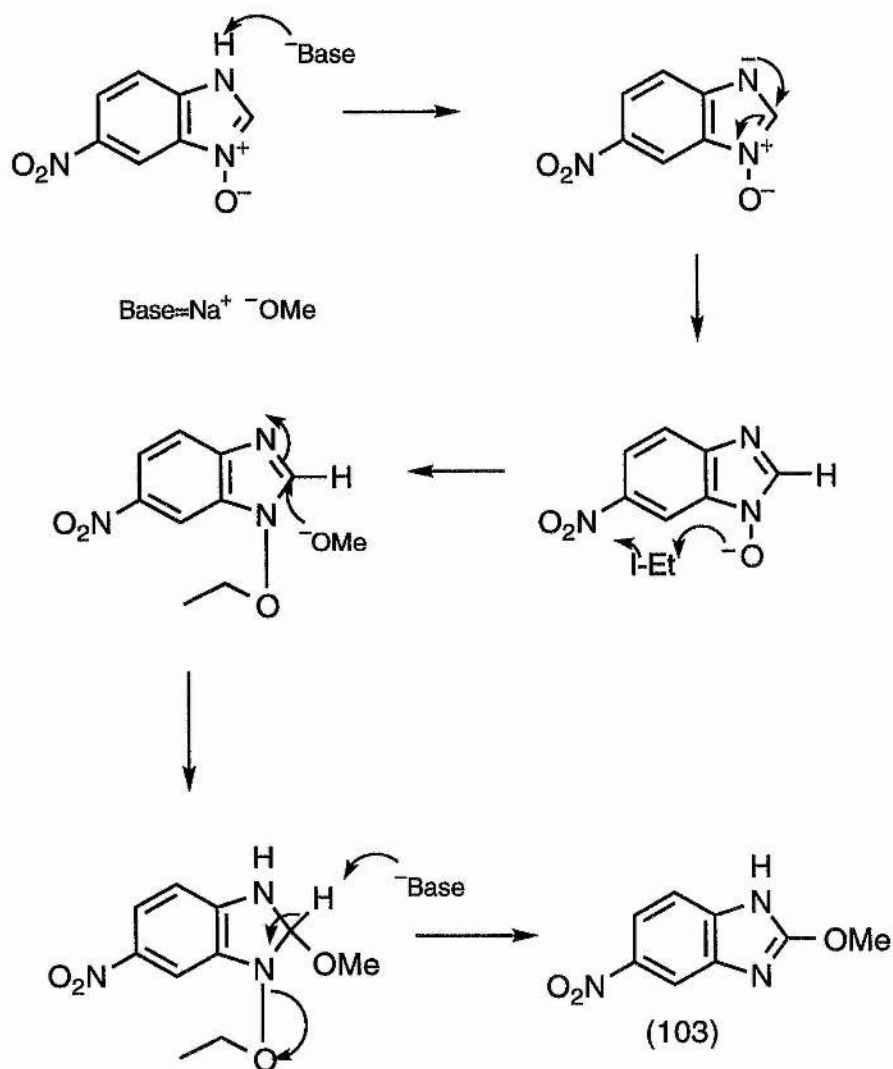
proved insoluble in the original ethanol solvent, hence the dipolar aprotic solvent was required to maintain the reactive species in solution.] Slight improvement in the yield was accomplished by adding the sodium ethoxide to a solution of the *N*-oxide in ethanol to create the anion, followed by removal of the solvent through evaporation then dissolution of the resultant solid in DMF before addition of the alkylating agent (ethyl iodide).



In further attempts to improve the alkylation yield, the addition of a two-fold excess of base resulted in the isolation of an unexpected product which could be explained by a rather interesting rearrangement<sup>80</sup> (see mechanism below). Alkylation initially occurs, as normal, at the more nucleophilic oxygen site following the addition of the first equivalent of base and the ethyl iodide; however upon addition of a second equivalent of base which attacks nucleophilically at C-2 and results in the subsequent elimination of the ethoxy group



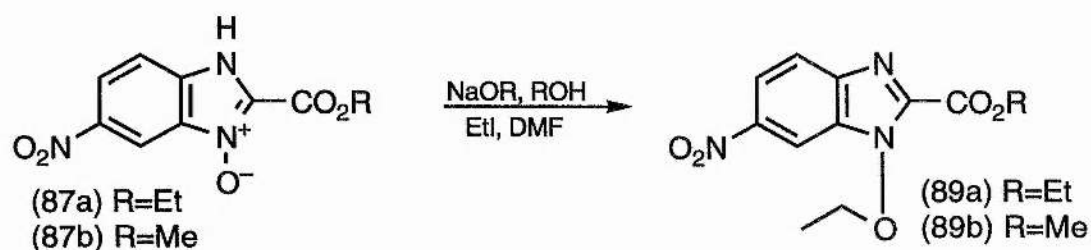
from N-1 giving the final product, 2-methoxy-5-nitrobenzimidazole (103), which was characterised by  $^1\text{H}$  nmr.



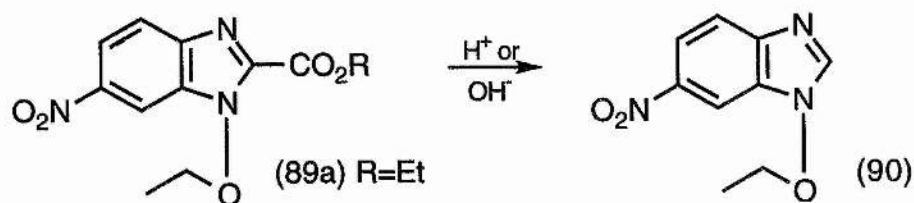
Although the addition of a two-fold excess of base resulted in elimination of the alkoxy group, one equivalent of base did not result in full deprotonation, inasmuch as peaks representing starting material were still present in the aromatic region of the  $^1\text{H}$  nmr spectrum of the final product. Addition of a slight excess of alkylating agent and base (each 1.1 equivalent) did little to improve the yield, with unreacted starting material still present in the final product, hence the reaction still not going to completion. The unreacted starting

material was removed by washing the organic phase of the reaction mixture with aqueous sodium hydroxide, via the formation of the water-soluble sodium salt of the benzimidazole *N*-oxide and its removal in the aqueous layer. This produced a product free from starting material according to the  $^1\text{H}$  nmr spectrum.

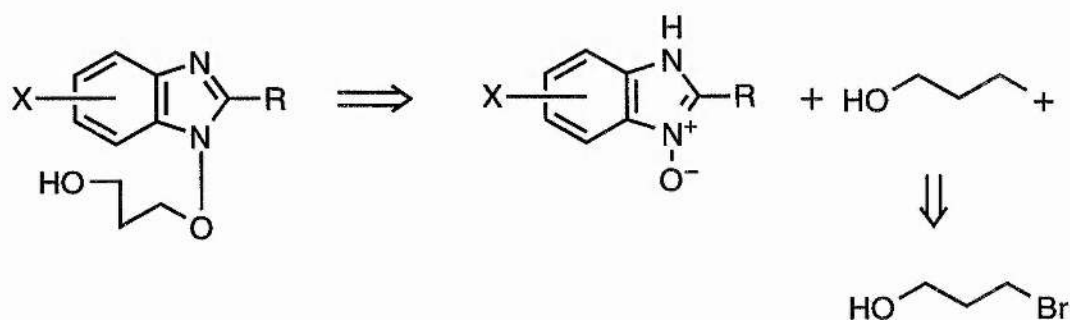
With the slightly improved alkylation conditions providing the desired product in reasonable yields, alkylation of both esters (87a) and (87b) was next undertaken (see scheme 2.1, page 28). Although no heating was applied to the reaction, it was debatable if the ester would withstand the reaction conditions. In the case of the ethyl ester (87a) the expected product (89a) was isolated in a reaction with sodium ethoxide and ethyl iodide in DMF; with the methyl ester, using sodium ethoxide as the base, exchange occurred between the ester and the ethoxide anion and this resulted in a mixture of ethyl and methyl esters. The use of sodium methoxide as the base in the presence of the methyl ester, prevented any such exchange.



With the alkylation in the presence of the ester groups having proved successful, hydrolysis/decarboxylation remained the only stage left to complete all the alternative routes prior to the final reductive stage. As no difference in the reactivity of the esters had been found so far, the acid and base hydrolyses were carried out only on the ethyl ester (89a). Acid hydrolysis of (89a) yielded a high-melting solid with the correct  $^1\text{H}$  nmr but with an  $\text{M}^+$  at  $m/z$  208 which corresponded to the hydrochloride of the desired product. Basic hydrolysis of the ester (89a) in sodium hydroxide solution (on addition of the hydroxide to the reactant, the characteristic deep red colour of the anion in solution was immediately visible) furnished the desired free *N*-oxide (90) after heating at  $50^\circ\text{C}$  for 2 hours.



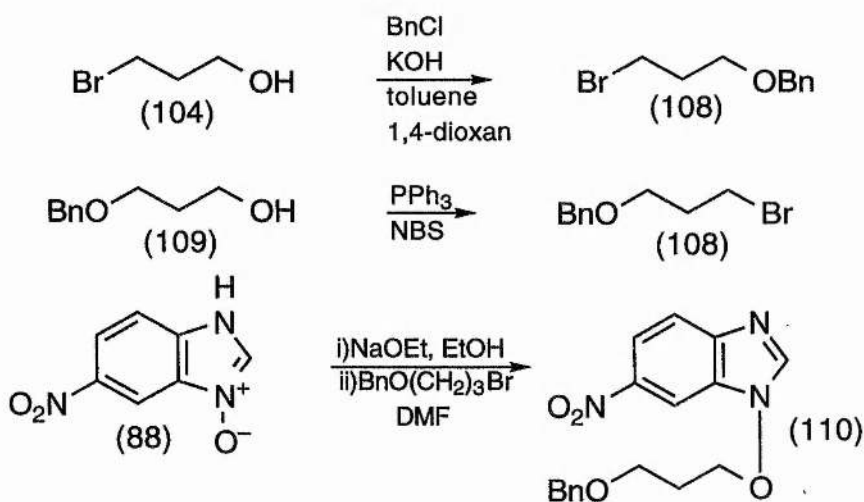
Attempts were then made to effect hydroxyalkylation of 5-nitrobenzimidazole 3-oxide with the inexpensive and commercially available 3-bromopropan-1-ol while maintaining the previously used reaction conditions. This would furnish a benzimidazole with a more complex acyclic side chain; equivalent in length to that of acyclovir (21) (see chapter 1, p11) but possessing the N-O-C linkage between the base and side chain similar to that of BRL 44385 (26) (see chapter 1, p13).



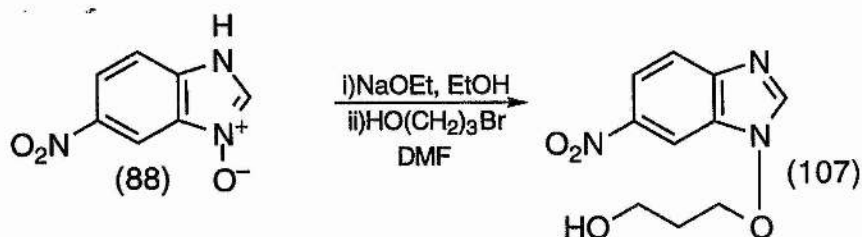
The hydroxyalkylation with 3-bromopropan-1-ol was initially attempted with the hydroxyl group protected as its 2-tetrahydropyranyl ether in order to avoid any unwanted side reactions of the hydroxyl group involving C-2, further alkylating agent, the nitro group or the amino group subsequent to reduction of the nitro group. Protection of the hydroxyl group as its tetrahydropyranyl ether was chosen due to its stability under a variety of conditions, including catalytic reduction, the ease of handling of dihydropyran and the mildness and efficiency of the conditions required to introduce the ether and furthermore to regenerate the alcohol<sup>83</sup> at the appropriate stage.



was investigated in order that hydrogenolysis of the benzyl group might occur in parallel with the reduction of the nitro group, so disposing of the need for a separate deprotection step. Two possible methods for the synthesis of 1-(benzyloxy)-3-bromopropane (108) were investigated. The first benzylation was developed from the method of Michelson and Todd<sup>85</sup>, originally used in the protection of sugars, however the yield was very poor and returned only enough of the product (108) for characterisation. The second method<sup>86</sup> proved a little more successful and involved a slightly different approach, the bromination of 1-(benzyloxy)-3-propanol (109) with *N*-bromosuccinimide and triphenylphosphine to provide the desired product (108) in more appreciable quantity. The reaction of the 3-bromo-1-benzyloxypropane (108) with 5-nitrobenzimidazole 3-oxide (88) gave the desired product (110) although in a very poor yield. Again the product (110) proved very difficult to purify and was not isolated in its pure form.



As this type of *O*-alkylation had been partially successful the reaction between 5-nitrobenzimidazole 3-oxide (88) and unprotected 3-bromo-1-propanol (104) was then attempted. When the reaction was carried out the tlc was seen to be more complex than those previously observed, but the major and only isolated product was the desired 1-(3-hydroxypropoxy)-6-nitrobenzimidazole (107), free from contaminants and judged to be pure spectroscopically. The good yield obtained implied that the reaction was substantially free of side reactions.



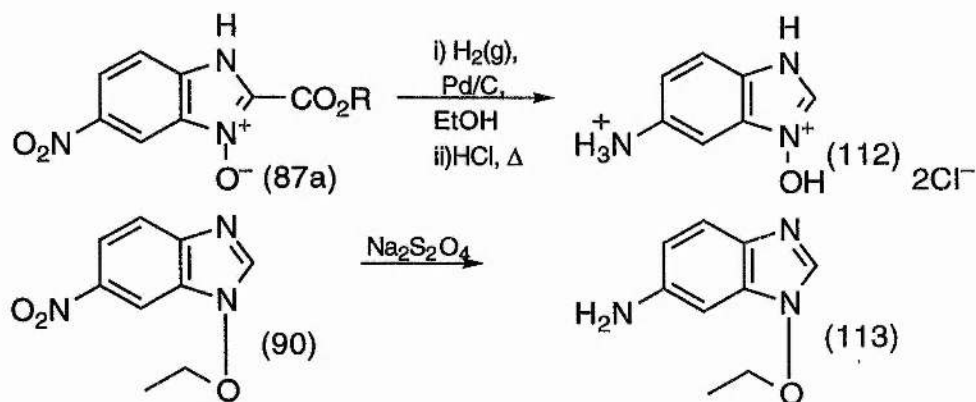
In the reaction of the unprotected 3-bromopropan-1-ol (104) with ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a), the possibility of unfavourable side reactions involving the hydroxyl group still existed. A further alteration to the reaction procedure was introduced in this reaction: potassium carbonate was used as the base instead of sodium ethoxide in an effort to improve the yield. This was a great success: potassium carbonate proved to be a far superior base, furnished the product (111) in a greater yield from a cleaner reaction, and disposed of the need to deprotonate the benzimidazole *N*-oxide in a separate step.



The (hydroxy)alkylation (using ethyl iodide and both 3-bromopropan-1-ol and its hydroxy-protected counterparts) was successful for both 5-nitrobenzimidazole 3-oxide (88) and its 2-carboxylate esters (87a/b). The only remaining step was the reduction of the nitro group to an amino group to produce the desired guanine functionality and hence a nucleoside analogue.

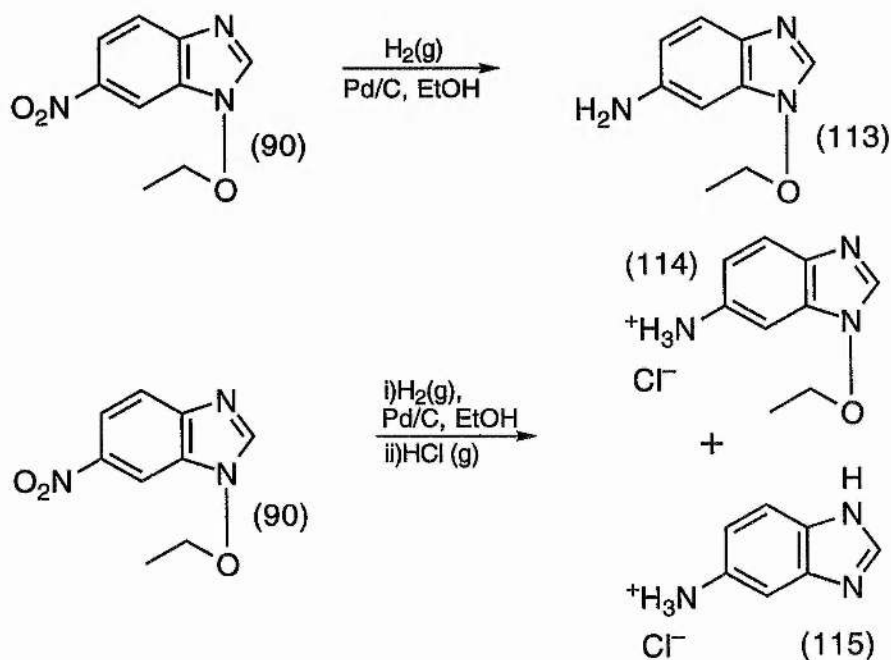
## 2.4 Attempted reductions of the nitro functionality

The first attempt at reduction of the nitro group was carried out on 1-ethoxy-6-nitrobenzimidazole (90), a model system, before the more complex hydroxypropoxy systems. 5-Aminobenzimidazole 3-oxide (112) had been produced previously<sup>87</sup> by catalytic hydrogenation. The synthesis however involved the catalytic hydrogenation of the ester (87a), followed by hydrolysis/decarboxylation, due to the insolubility of the nitro compound (88) in typical hydrogenation solvents. The final product (112), reached via acid hydrolysis, was isolated as the dihydrochloride. Work by the author<sup>88</sup> had previously resulted in the spectroscopic detection, but not isolation, of 6-amino-1-ethoxybenzimidazole (113), but time constraints had prevented further investigation and the reaction was only attempted once. The reducing agent used was sodium dithionite<sup>89</sup> and although the reduction had been successful (by <sup>1</sup>H nmr/mass spectrum), the yield had been very poor.



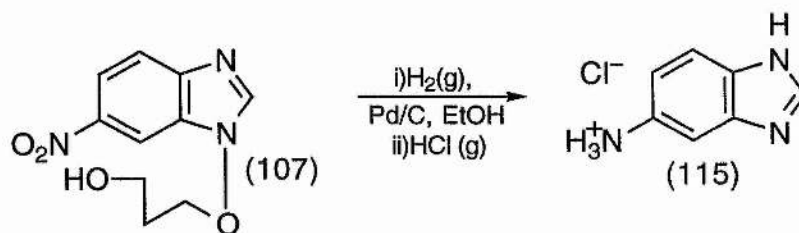
Catalytic hydrogenation was the first method employed in the attempted reduction of the nitro group of compound (90); using 5% palladium on carbon as the catalyst, as in the previous<sup>87</sup> successful reduction of (87a). The product (113), isolated in low yield, was purified on a column of silica and had the correct <sup>1</sup>H nmr, but proved unstable in air, the light coloured product darkening over a few days. With this knowledge, the catalytic hydrogenation was reattempted but the final solution treated with hydrogen chloride gas to

prompt the crystallisation of hydrochloride or dihydrochloride salt of 6-amino-1-ethoxybenzimidazole (113). This protocol led to the formation of a white precipitate which was collected. A second crop of crystals was also isolated from the supernatant liquid. Spectroscopic analysis of the two products showed that the first product was 5-aminobenzimidazole (115), and the second (in very low yield) was 6-amino-1-ethoxybenzimidazole (114), both isolated as hydrochlorides. This result clarified the reason for isolation of poor yields in the previous attempts; hydrogenolysis of the N-O bond had occurred in addition to the reduction of the nitro group, to furnish ethanol and the water-soluble 5-aminobenzimidazole as the products.



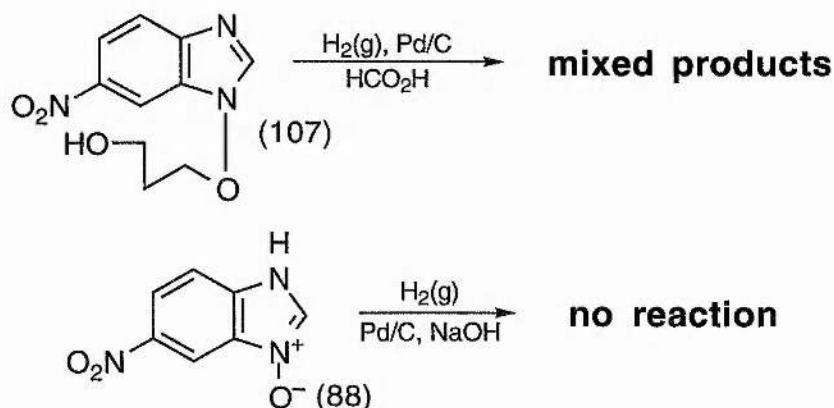
Further attempts at the reduction of the nitro group were undertaken using the same conditions but with 1-(3-hydroxypropoxy)-6-nitrobenzimidazole (107). However the only product obtained was 5-aminobenzimidazole (115) which was isolated as its hydrochloride: this crystallised from solution after removal of the catalyst and saturation with hydrogen chloride gas. The reductive cleavage of the N-O bond apparently occurred to a greater extent with the hydroxypropoxy side chain. The reason for this is not immediately obvious.





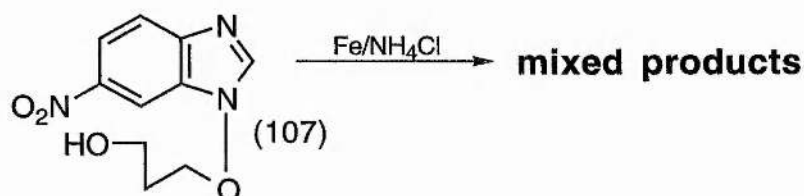
In the work of Harnden<sup>43</sup> *et al.* directed towards the synthesis of the drug Famvir (see chapter 1, p13) hydrogenolysis of a benzyl group in the presence of an N-O-C linkage between a hydroxypropoxy group and an adenine derivative was accomplished successfully using hydrogen and palladium on carbon catalyst in formic acid.

These aforementioned conditions were applied to the reduction of 1-(3-hydroxypropoxy)-6-nitrobenzimidazole (107). Yet again however, hydrogenolysis of the N-O-C linkage occurred, with no evidence of the desired product in the <sup>1</sup>H nmr spectrum, the aromatic region only showing the protons associated with 5-aminobenzimidazole. The final hydrogenative method attempted involved the dissolution of the nitrobenzimidazole oxide (88) in sodium hydroxide solution due to its insolubility in normal hydrogenative solvents. The attempted hydrogenation of the nitro group under these conditions with palladium/carbon catalyst produced no reaction; the starting material (88) was returned unchanged.

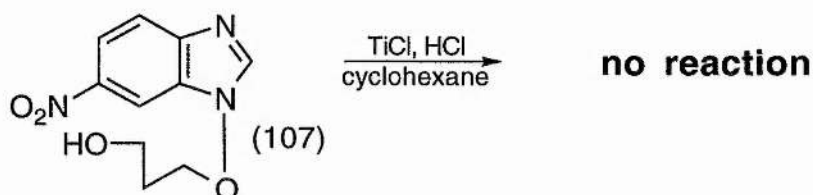


With no success using hydrogenative methods for the required reduction, other more selective and milder conditions were considered. Iron is a metal widely used in the

reduction of nitro groups to amines, however other susceptible groups are often affected and the N-O-C link has already proved to be easily cleaved under hydrogenative conditions. It has been reported<sup>90</sup> that the iron-ammonium chloride system is a mild reductive protocol for the production of amine products in quantitative yields from nitro compounds in the presence of reducible groups such as nitriles and ketones. Application of the literature procedure to 1-(3-hydroxypropoxy)-6-nitrobenzimidazole (107), however, resulted in the detection of no discernable product. The <sup>1</sup>H nmr showed no evidence of either unreacted starting material or products, and the tlc showed a variety of products; the method was abandoned, the conditions presumably being too harsh and resulting in the decomposition of the starting material or products.



An extremely mild method<sup>91</sup> for the reduction of aromatic nitro compounds to aromatic amines and utilises titanium trichloride in hydrochloric acid as the reductant. The starting material (107) had already proved stable under prolonged heating in concentrated hydrochloric acid and the proposed conditions were certainly less drastic than those associated with tin or iron in hydrochloric acid.



Unfortunately in practice, these conditions also proved ineffectual as a method for the reduction of the nitro group and returned the starting material unchanged, with the N-O-C bonds intact. This did show however, that reductive conditions did not necessarily

to the cleavage of the N-O bond. With no success imminent however in the search for a protocol for the reduction of the nitro functionality in the presence of the N-O-C linkage, it was decided that the synthetic route ought to be redesigned to dispose of the need for such a reaction.

## 2.5 A non-reductive route to the desired guanosine analogue

In theory, several possibilities were available which could be manipulated to provide an amine and lead to the desired nucleoside analogues: i) the reduction of the nitro group before *O*-alkylation of the *N*-oxide, ii) the production of a suitably functionalised benzimidazole *N*-oxide which could furnish the amine upon reaction with a nitrogen nucleophile, or iii) commencement of the synthetic route with the nitrogen at the correct oxidation level and suitable protection of the amine.

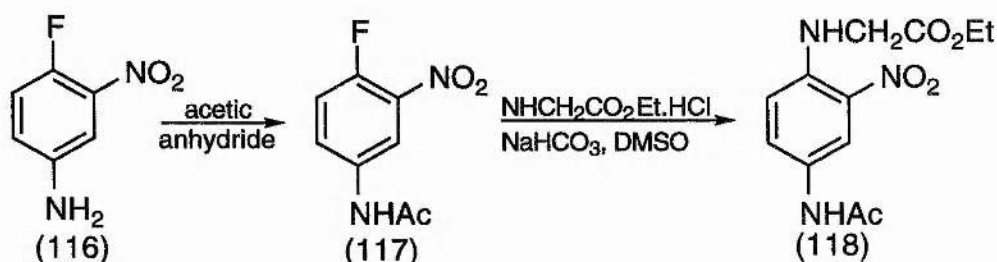
The reduction of the nitro group at a different stage of the synthesis was immediately discounted due to the difficulties incurred by previous workers<sup>87</sup> (see p41) in trying to isolate species in pure form. Secondly, alkylation specifically on the oxygen atom may then become a problem as the amine could also be susceptible to alkylation; and finally, reaction of the 2-carboxylate ester and the amine functionalities may introduce the possibility of self-condensation.

The second possibility involving functional group transformation via nucleophilic displacement was also disregarded, as such a reactive position could prove problematic throughout the synthetic route.

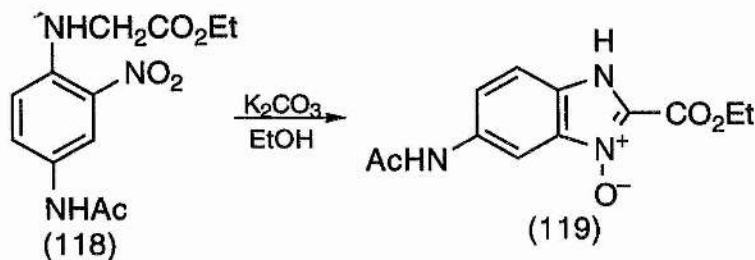
Hence the most promising option was the protection of the amino functionality from the outset, beginning with the nitrogen at the correct oxidation level, and introducing two extra steps to the synthesis: a protective step at the beginning and a deprotective step at the end. A protected amino group, although not electron withdrawing by nature, is electron withdrawing by inductive effect, hence the ring should not differ too much in reactivity from the dinitrophenylglycine in the cyclisation step. In view of previous work,<sup>81,82,87</sup>

protection of the potential 5-amino group as its acetamide was considered to be the most straightforward route.

The amino group of 4-fluoro-3-nitroaniline (116) was protected through its reaction with acetic anhydride, yielding 4-fluoro-3-nitroacetanilide (117). This was in turn reacted with glycine ethyl ester hydrochloride and sodium bicarbonate in dimethyl sulphoxide, furnishing the nitrophenylglycine (118). The stronger reaction conditions (cf. chlorodinitrobenzene and nitrophenylglycine) were required to effect the nucleophilic substitution reaction in the absence of a second strongly electron-withdrawing substituent on the ring.

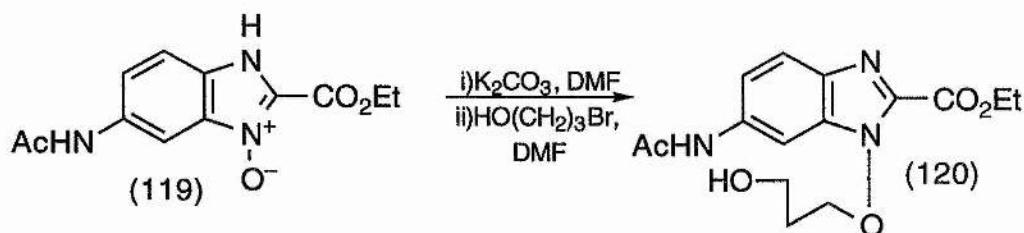


The cyclisation to ethyl 5-acetamidobenzimidazole-2-carboxylate 3-oxide (119) was effected under the now standard conditions of reflux in ethanol with potassium carbonate as the base. The protection of the amino group has a two-fold purpose; firstly the amino group is unable to participate in self condensation reactions with the carboxylate ester either before, during or after the cyclisation stage; and secondly, the strong electron-donating nature of the amino group would create difficulties in the reaction with the glycine nucleophile and also reduce the electrophilicity of the *ortho*-nitro group. Although ring substituent effects have not been systematically studied for intramolecular reactions involving nitro groups, it has been noted<sup>87</sup> that the presence of an amino group, even where there are two nitro groups present, prevents cyclisation, whereas with an acetamide the cyclisations proceed as expected.



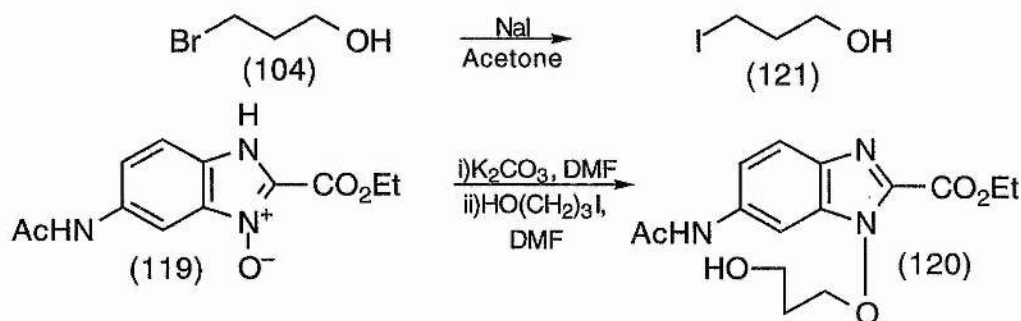
In the case of ethyl 5-acetamidobenzimidazole-2-carboxylate 3-oxide (119), the conditions required for hydrolysis/decarboxylation in concentrated acid would lead to the hydrolysis of the acetamide before the alkylation had been carried out, the foremost reason for the protection of the amine; *O*-alkylation of the benzimidazole oxide (119) was thus attempted prior to the removal of the ester group.

The alkylation of this benzimidazole (119) was effected in the usual way by treatment with potassium carbonate in DMF, but in this instance a characteristic colour change did not occur to indicate the presence of the anion in solution [as in the case of the 5-nitrobenzimidazole 3-oxide (88)]. A small degree of precipitation occurred but the precipitate was resolubilised by the addition of a further aliquot of DMF. After the addition of 3-bromopropan-1-ol (104) the desired product (120) was isolated, the hydroxyalkylation having occurred on the oxygen with no significant side reactions involving the ester or the acetamide. The reaction yields were rather disappointing, hence the reaction was reattempted, both at low temperature (0°C) over a greater time period to suppress side reactions, and also for a shorter period at higher temperature (~100°C) but these resulted in no discernable improvement in the reaction yields.



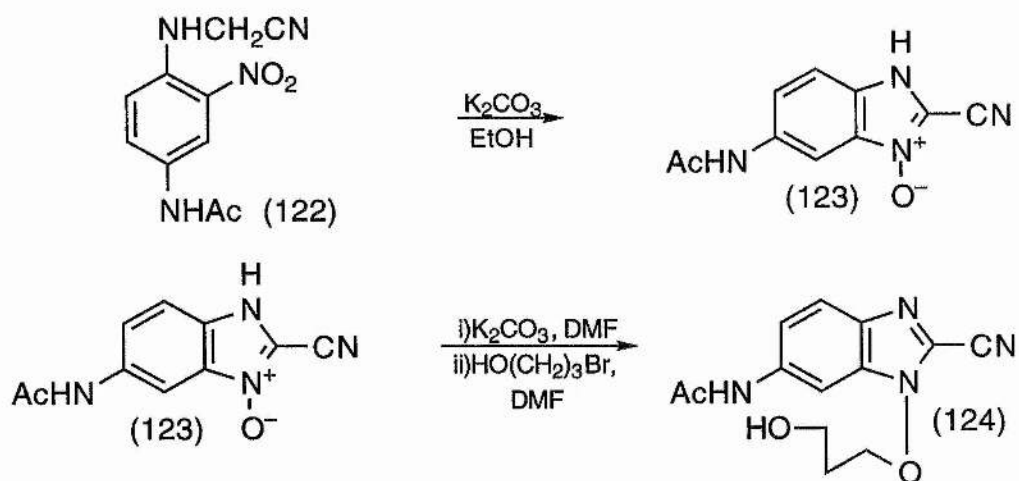
The effectiveness of bromide as a leaving group in the alkylation reaction was next brought under scrutiny. Using the Finkelstein reaction, 3-bromopropan-1-ol (104) was reacted with sodium iodide in acetone to yield 3-iodopropan-1-ol (121). Reaction times were increased from the literature procedure<sup>92,93,94</sup> to ensure an adequate yield of the 3-iodopropan-1-ol (121). Distillation of the reaction mixture separated the product from unreacted starting materials. Exchange of the iodide for bromide resulted in a drastic shift in the  $^{13}\text{C}$  signal for the methylene group attached to the halide. There was a 30ppm upfield shift in the halogenomethylene resonance, probably due to the shielding effects of the iodine *p* and *d* orbitals.

The use of 3-iodopropan-1-ol (121) in the hydroxyalkylation of ethyl 5-acetamidobenzimidazole-2-carboxylate 3-oxide (119) using potassium carbonate in DMF did not produce a notable increase in the yield, not justifying the extra preparative step in the reaction route.



Although, thus far in all this synthetic work, the use of substituted *N*-cyanomethyl-*o*-nitroanilines as starting materials had been avoided due to the cyanomethylation step requiring the use of potassium cyanide, old stocks of 4-acetamido-*N*-cyanomethyl-2-nitroaniline (122) were available for cyclisation. The reaction was carried out for a direct comparison with that of the ethyl ester in both the cyclisation and alkylation stages. The cyclisation, using potassium carbonate as the base, proceeded smoothly, yielding the product (123) but in a fairly low yield; however the hydroxyalkylation of 5-acetamido-2-

cyanobenzimidazole 3-oxide proceeded very well and furnished the product (124) in high yield- in fact, the best yield of any of the alkylations attempted.



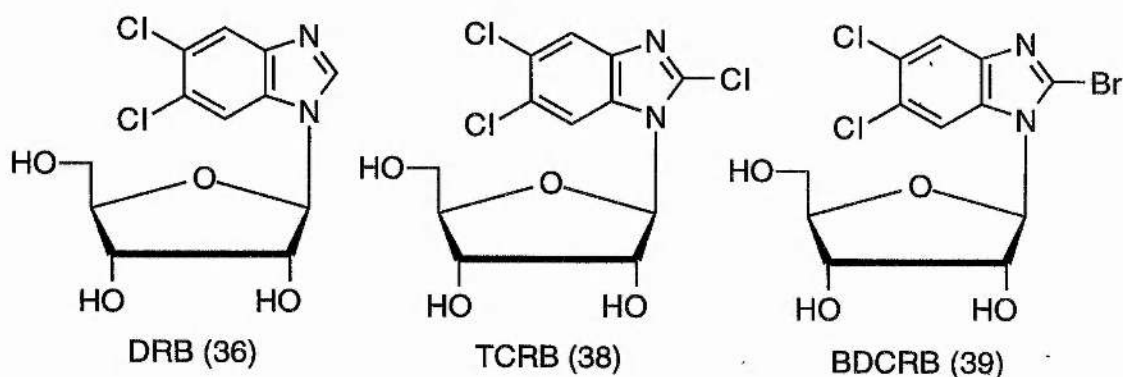
A selection of these alkoxybenzimidazoles was subjected to antiviral testing. This is covered in chapter 5.

## Chapter 3

### Other benzimidazole N-oxides

#### 3.1 Introduction

Recent work carried out by Townsend *et al.*<sup>53,54</sup> in Michigan into benzimidazole ribonucleosides resulted in the discovery of the activity against HCMV of two compounds, TCRB (38) and BDCRB (39), both derivatives of DRB<sup>51</sup> (36) (see chapter 1, p16). A great variety of nucleoside analogues were initially synthesised before the highly potent and selective compounds (38) and (39) were discovered. With only one exception<sup>95</sup>, derivatives of the initial compound, DRB (36), with modification to the benzene ring halogens or movement of the halogens to different positions on the benzene ring resulted in poorer antiviral activity. Replacement of the ribose sugar with other sugars producing sugar-modified analogues also resulted in weaker antiviral activity except in the cases of both the xylo and lyxo analogues which possessed activity against HSV1 and HSV2<sup>95</sup>.

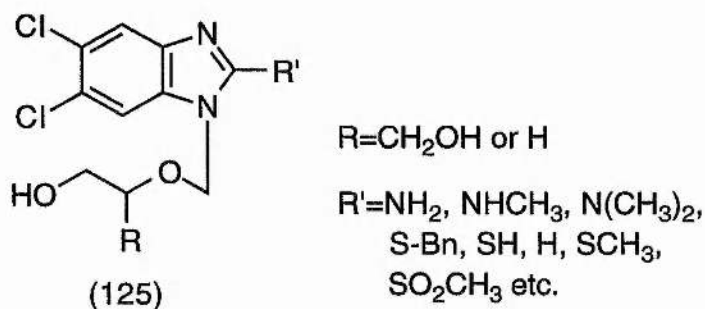


Analogues of (38) and (39) in which the ribose sugar was replaced by an acyclic moiety at N-1 [analogous to acyclovir (21) (chapter 1, p11) or ganciclovir (23) (chapter 1,



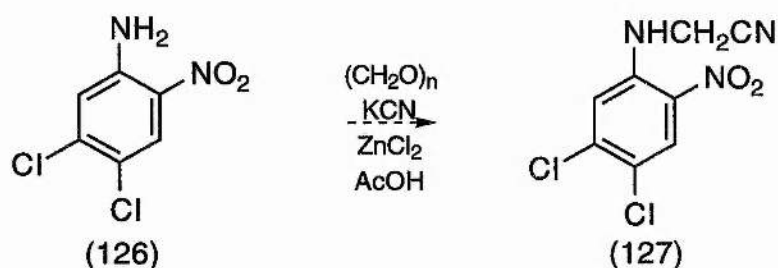
p12), two of the commercially available treatments for HCMV] also resulted in compounds (125, R=H or CH<sub>2</sub>OH) which were either only weakly active or inactive against HCMV or HSV1<sup>96</sup>.

The final variation in structure which was investigated was the atom or group at C-2 (125, R' is a variety of substituents). The introduction of a chlorine or bromine at C-2 resulted in a notable improvement in the activity of (38) and (39) against HCMV and selectivity relative to DRB. Several other substituents were investigated at C-2 but none showed an improvement in activity over (38) and (39). The lone heterocycles of the benzimidazole nucleosides were also tested for antiviral activity and showed promise in plaque reduction assays, but the activity was not well separated from toxicity<sup>53,54,96</sup>.

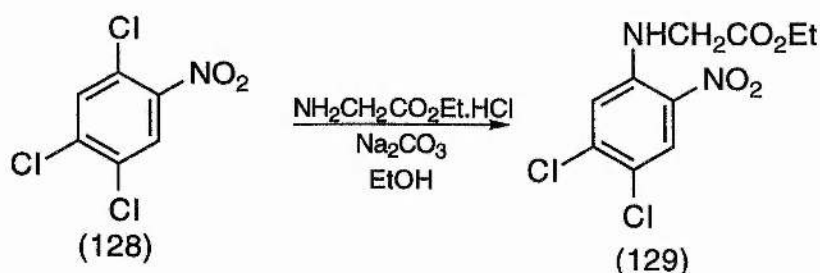


These observations indicated that the requirements for HCMV activity for benzimidazole ribonucleosides, especially at C-2, are extremely narrow and specific. This work prompted an interest in the synthesis of dichlorobenzimidazole *N*-oxides; research into these compounds had never before been undertaken. The synthesis and *O*-alkylation of 5,6-dichlorobenzimidazole 3-oxide was of particular interest. The two possible synthetic precursors of this type of benzimidazole oxide were the ester (129) and the nitrile (127). It was expected that there might be difficulties in the synthesis of (127) from 4,5-dichloro-2-nitroaniline (126), paraformaldehyde, potassium cyanide and zinc chloride<sup>73</sup>. The quantity of zinc chloride required varies with the basicity of the amine<sup>73</sup>, a large excess (7-8 mol.

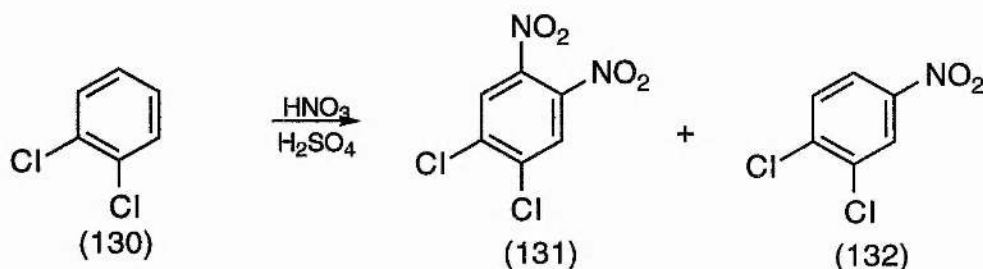
equiv.) being required even for monochloro-nitroanilines, and so there was the possibility that an even larger proportion might be necessary in the case of dichloronitroanilines. This method was therefore not investigated further and attention was concentrated on the synthesis of the ester (129).



In the reaction of 1,2,4-trichloro-5-nitrobenzene (128) with an uncharged nitrogen nucleophile, there remained the theoretical possibility that the *ortho*-, *para*- or indeed both chlorines may be displaced by the glycine. In any case, such a substitution was known to occur and displace the desired *ortho*-chlorine as in the reaction of 1,2,4-trichloro-5-nitrobenzene (128) with hydrazine hydrate to furnish 5,6-dichloro-1-hydroxybenzotriazole<sup>97</sup> (discussed in chapter 4). In such a reaction involving glycine, the reaction conditions used could not be as severe as those involved in the reaction of a simple nitrogen nucleophile such as hydrazine. The reaction of 1,2,4-trichloro-5-nitrobenzene (128) was investigated by Marr<sup>98</sup> and although the desired ester product (129) was furnished, the nitrogen nucleophile displacing the *ortho*-chlorine, the yield was very poor. This was presumably due to the poor polarisability of the C-Cl bond and its much poorer lability compared to e.g. a fluorine<sup>99</sup>.

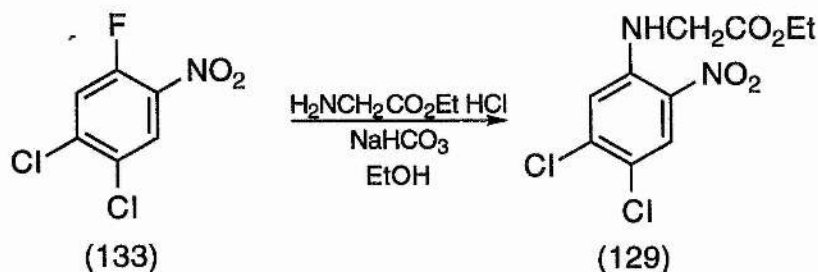


The use of a nitro group as a suitable leaving group was also investigated by Marr<sup>99</sup>. *ortho*-Dichlorobenzene (130) was dinitrated<sup>100</sup> to give a mixture of products including the desired 1,2-dichloro-4,5-dinitrobenzene (131). It was reported that nitro groups may be substituted with nitrogen nucleophiles<sup>101</sup>. Although such a substitution reaction was investigated by Marr<sup>98</sup> and the desired dichloronitrophenylglycine ethyl ester isolated, it was only isolated in a very poor yield; the route was not investigated further due to both the poor yield of the substitution reaction and the mixture of products isolated from the dinitration of *o*-dichlorobenzene (130).

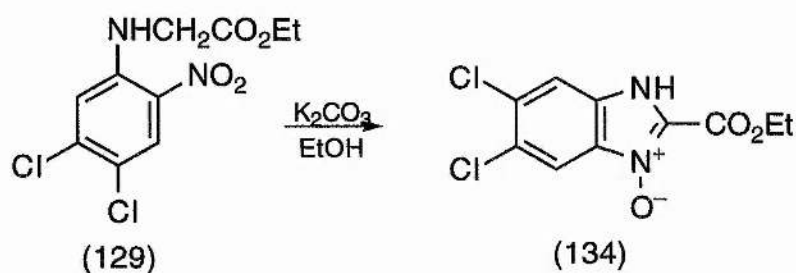


### 3.2 Synthesis of 5,6-dichlorobenzimidazole 3-oxide

The route followed to the dichlorobenzimidazole *N*-oxide was similar to that undertaken for the synthesis of 5-nitrobenzimidazole 3-oxide; the cyclisation of a nitrophenylglycine derivative to the benzimidazole *N*-oxide with functionality at C-2 was followed by either hydrolysis/decarboxylation or by *O*-alkylation depending on whether or not the ester group was required at C-2 of the final product. Commercially available 1,2-dichloro-4-fluoro-5-nitrobenzene (133) was heated in ethanol with glycine ethyl ester hydrochloride and sodium bicarbonate to furnish the desired 1,2-dichloro-4-nitrophenylglycine ethyl ester (129) in good yield.



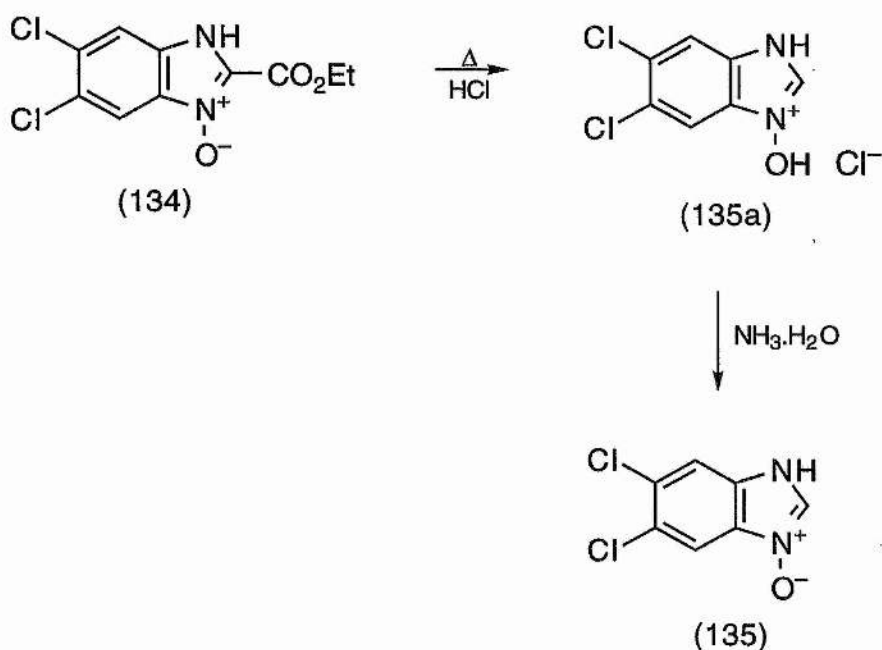
The dichloronitrophenylglycine ester (129) was then cyclised in ethanol with potassium carbonate to yield ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134) using the now familiar conditions. The product was isolated in a rather disappointing yield (ca. 50%) but was found to be pure spectroscopically, and no optimisation of the reaction conditions was undertaken. However, one of the later attempts at this reaction was left overnight before the work-up procedure was carried out, and upon analysis of the crude product by  $^1\text{H}$  nmr it was found that a substantial amount of hydrolysis/decarboxylation had occurred; a downfield resonance for H-2 was apparent and the integration of the upfield aliphatic resonances was incorrect. It was presumed that if the base used for the cyclisation was a good enough nucleophile, then attack at the ester was possible and following ester hydrolysis, decarboxylation would be spontaneous<sup>74</sup> and furnish a mixture of products (134) and (135). This hydrolysis/decarboxylation of the C-2 functionality by a nucleophilic base had not been previously noticed and was a distinct possibility in all such reactions. The poor yields of earlier cyclisations were probably due to this fact. The crude mixture of products (134) and (135) was heated under reflux in an excess of concentrated hydrochloric acid to complete the hydrolysis/decarboxylation and this procedure<sup>73</sup> led to the isolation of the hydrochloride (135a) in a good overall yield.



As discussed in the introduction to this chapter (p50 and p51), the benzimidazole ribonucleosides synthesised by Townsend *et al.*<sup>53,54,96</sup> required functionality at C-2 for the maintenance of activity against HCMV; although many substituents were experimented with, no alkyl esters were investigated.

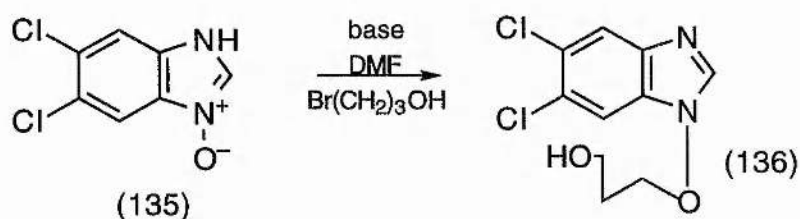
Two possible routes were available in the synthesis. The ester (134) could be *O*-alkylated to yield a product with functionality at C-2. Hydrolysis/decarboxylation of the ester (134) to remove the C-2 functionality followed by *O*-alkylation was the alternative. Both possibilities were investigated in order to highlight any differences in activity due to the presence or absence of functionality at C-2.

Ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134) was heated under reflux in concentrated hydrochloric acid and the 2-unsubstituted *N*-oxide isolated as its hydrochloride (135a). The free *N*-oxide (135) was obtained after a simple work-up in ammonia solution and identified by the upfield shift of the H-2 resonance relative to the chemical shift of H-2 in the hydrochloride (135a)<sup>73</sup>.

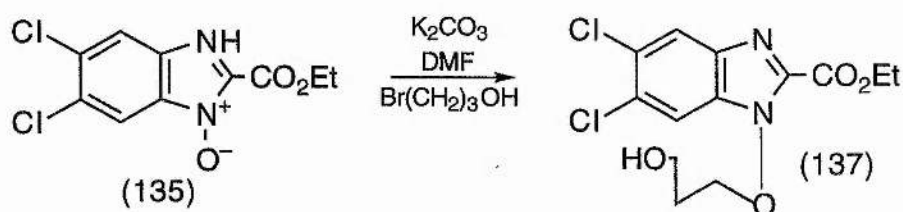


### 3.3 *O*-Alkylation of the dichlorobenzimidazole oxides

*O*-Alkylation of the *N*-oxide (135) was carried out next using 3-bromopropan-1-ol in the first instance; the use of ethyl iodide in preliminary attempts and protection of the hydroxyl group of 3-bromopropan-1-ol were previously proved to be unnecessary (see chapter 2, p39). Sodium ethoxide was initially used as the base in the *O*-alkylation but as in the case of 5-nitrobenzimidazole 3-oxide (chapter 2, p40) the yields were very poor. The use instead of potassium carbonate greatly improved the *O*-alkylation reaction yield. An attempt to furnish (136) using triethylamine as the base also resulted in a poor yield.



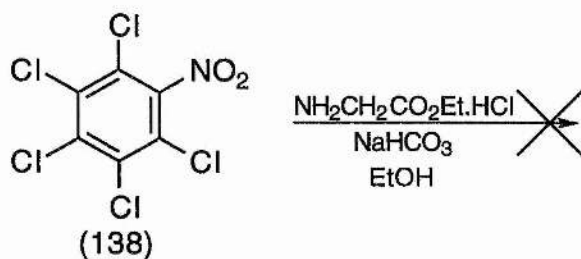
The corresponding *O*-alkylation of the ester (134) using potassium carbonate as the base furnished the desired product (137) in 43% yield. This reaction was also attempted at low temperature (0°C) and high temperature (90°C): the reaction at high temperature resulted in the partial hydrolysis/decarboxylation at C-2 and furnished a mixture of products (136) and (137). The low temperature reaction furnished the expected product (137) as normal but with no improvement in yield.



The mixture of carboxylated (137) and decarboxylated (136) material obtained for the high temperature *O*-alkylation was heated under reflux in concentrated hydrochloric acid to yield the entirely hydrolysed/decarboxylated material (136). In this type of reaction there was no evidence of any cleavage of the N-O-C linkage under the hydrolytic conditions. There was also no evidence of protonation at N-3, hence isolation of hydrochloride salts of the product. In all cases where hydrolysis/decarboxylation was carried out on an *O*-alkylated benzimidazole oxide there was no evidence of protonation at N-3. The chemical shift of H-2 was always in the more upfield region of the spectrum as expected when comparing the chemical shifts of H-2 in free benzimidazole *N*-oxides relative to H-2 in the hydrochloride salts. The low melting points of all the *O*-alkylated benzimidazoles indicated very little hydrogen-bonding between the molecules, cf. imidazole and imidazole *N*-oxide.

### 3.4 Polychloro benzimidazole oxides

The reaction of pentachloronitrobenzene (138) with glycine ethyl ester hydrochloride in a solution of ethanol and toluene using sodium bicarbonate as the base was attempted. Analysis of the crude reaction mixture by tlc and  $^1\text{H}$  nmr showed that no reaction had occurred. The reaction was pursued no further as the attempt had only been carried out due to the availability of the starting material in the laboratory. It was suspected that the reaction did not occur due to steric reasons.



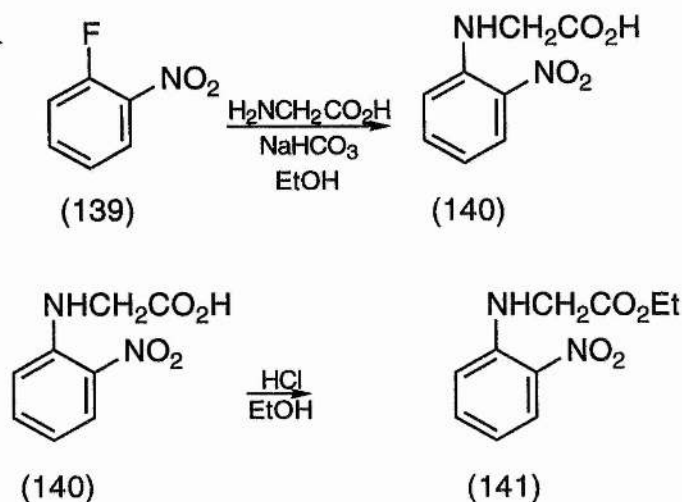
The products of particular biological interest and with similar functionality similar to Townsend's compounds are (136) and (137) and their activity against HSV1 is discussed in chapter 5.

### 3.5 Synthesis of benzimidazole 3-oxide

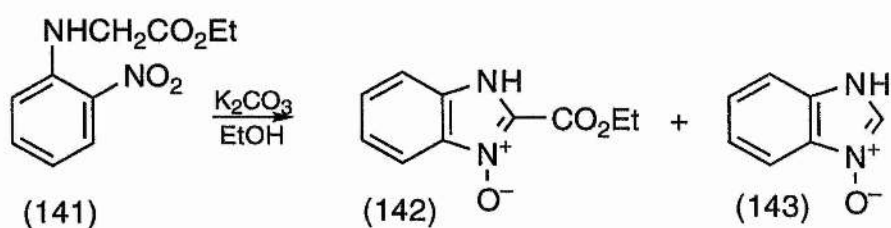
It was decided to synthesise the parent benzimidazole 3-oxide (143), unsubstituted on the benzene ring and at N-1, to act as a guide to the substituent effects on the activity of the compounds tested in an antiviral assay. The synthesis of benzimidazole 3-oxide was attempted using the conditions utilised in all the previous syntheses in this thesis. *o*-Fluoronitrobenzene was heated under reflux with glycine methyl ester hydrochloride and sodium bicarbonate in methanol but the major and only material isolated from the reaction mixture was unreacted starting material. In such reactions, cyclisation of the nitrophenylglycine ester formed may proceed under the conditions of the substitution reaction, and the cyclisation product may react further still. In this particular case it is far more convenient to react *o*-fluoronitrobenzene (139) with glycine and esterify in a separate step to enable complete cyclisation to be carried out when required and avoid the isolation of a complex mixture of products.

*o*-Fluoronitrobenzene (139) was heated under reflux with sodium bicarbonate and glycine to yield *o*-nitrophenylglycine (140). A small amount of the esterified product (141) was also isolated as a by-product. This was presumed to have occurred since the crude product (140), when dissolved in the recrystallisation solvent (ethanol), still contained some hydrochloric acid from the work-up procedure.

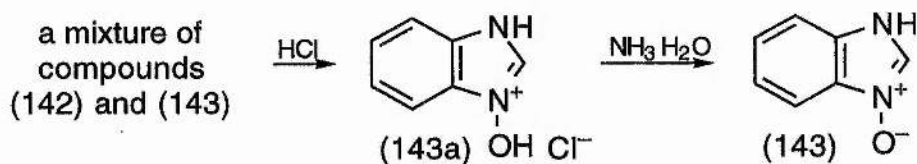




2-Nitrophenylglycine ethyl ester (141) was obtained from *o*-nitrophenylglycine (140) through esterification in acidic ethanol. The cyclisation to ethyl benzimidazole-2-carboxylate 3-oxide (142) was carried out, as previously perfected, with potassium carbonate. In the majority of attempts however, this reaction did not stop at the desired product but proceeded to the decarboxylated benzimidazole (143), hence furnishing a mixture of products. As previously mentioned (chapter 3, p54), the accompanying hydrolysis/ decarboxylation was due to the nucleophilic nature of the base used; an added complication in this particular case was the water solubility of the product of such a reaction: benzimidazole 3-oxide. The yield over the two stages of cyclisation and hydrolysis/ decarboxylation was reasonable. In only one case was the ester (142) isolated alone.



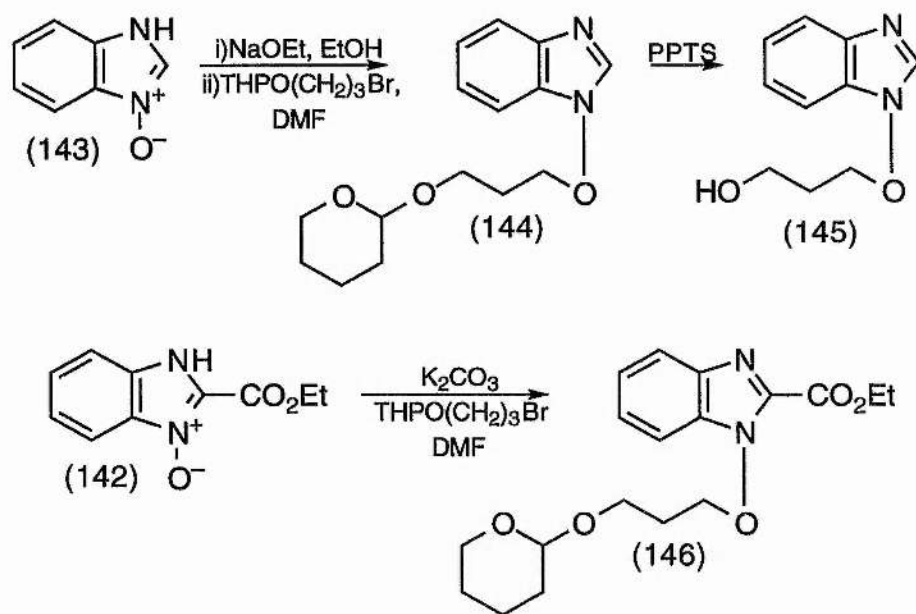
As has been mentioned previously, the ethyl benzimidazole-2-carboxylate 3-oxide (143) could either undergo *O*-alkylation or hydrolysis/decarboxylation first. Hydrolysis/decarboxylation will be dealt with initially. With the cyclisation reaction generally providing a mixture of products (142) and (143), the hydrolysis/decarboxylation was usually carried out on such a mixture. The hydrochloride (143a) was obtained in the first instance as the only product in the reaction and the parent benzimidazole oxide (143) isolated after a simple work-up in aqueous ammonia.



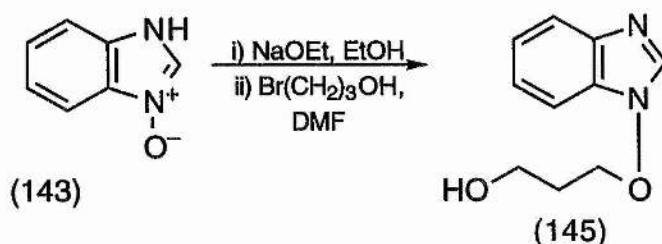
### 3.6 *O*-Alkylation of the benzimidazole oxide

In the early *O*-alkylations of the benzimidazole 3-oxide (143), as in the case of 5-nitrobenzimidazole 3-oxide (chapter 2, p38), protection of the hydroxyl group of 3-bromopropan-1-ol (104) was thought to be necessary. In the reaction of benzimidazole 3-oxide (143) with the THP ether (105) and sodium ethoxide, the desired product (144) was detected and isolated, and purification attempted on a column of silica. This method of purification was unsuccessful and the attempted distillation of the product at reduced pressure was also unsuccessful as the alkylated benzimidazole (144) proved to be too thermally unstable and decomposed. The purification prior to the removal of the THP protective group was not investigated further as deprotection was necessary before the final product (145) was reached and purification could then be carried out more thoroughly. The reaction of (142) was undertaken using potassium carbonate as the base, but once again the

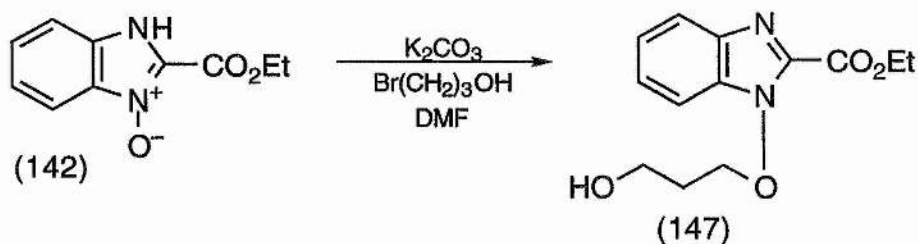
product (146) was only isolated in a very small yield; this was presumed to be due to steric factors. The THP protective group was removed using pyridinium *p*-toluenesulphonate (PPTS) as the catalyst. Although the product (145) was isolated and the correct  $^1\text{H}$  and  $^{13}\text{C}$  nmr resonances identified, (145) could not be obtained in pure form, proving too polar to purify on a column of silica and too thermally unstable, like its THP protected precursor (144), for distillation at reduced pressure.



On finding that a protective group for the hydroxyl function of 3-bromopropan-1-ol was an unnecessary precaution, indeed possibly a hindrance on steric grounds (all the *O*-alkylations involving the THP ether gave poor yields of products), the reaction was carried out with unprotected 3-bromopropan-1-ol (104). Initially sodium ethoxide was used as the base, where further attempts to purify the crude isolate by distillation at low pressure also led to decomposition, and as before, the replacement of sodium ethoxide with potassium carbonate improved the yields of (145) but it could still not be isolated in a pure enough form for use in the antiviral assay.



The reaction of ethyl benzimidazole-2-carboxylate 3-oxide (142) with 3-bromopropan-1-ol (104) with potassium carbonate as the base furnished the desired product (147) in good yield. There was no evidence of loss of the functionality from C-2 under the *O*-alkylation conditions. Hydrolysis/decarboxylation of (147) was not attempted since (145) could not be isolated in a sufficiently pure form.

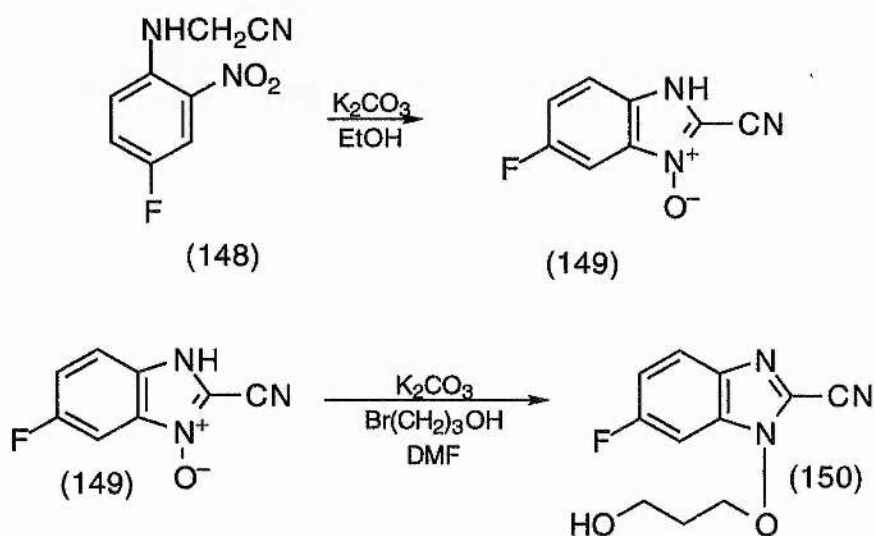


Ethyl 1-(3-hydroxypropoxy)benzimidazole-2-carboxylate (147) was of particular interest for its activity against HSV1 and is discussed in chapter 5 but the C-2 unsubstituted 1-(3-hydroxypropoxy)benzimidazole (145) was not submitted for antiviral testing as any activity discovered could not be attributed solely to the desired compound as the sample was contaminated with unknown impurities.

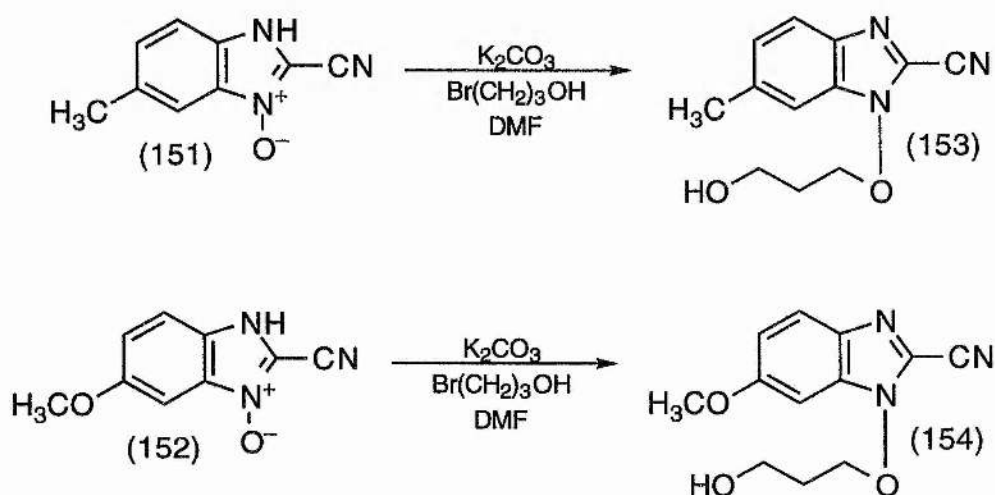
### 3.7 *O*-Alkylation of other substituted benzimidazole oxides

A few benzimidazole *N*-oxides with a variety of substituents on the benzene ring, and a number of their synthetic precursors, were already available in the laboratory in sufficient quantities to enable the investigation of their *O*-alkylation. Many of these compounds had been synthesised via the cyclisation of *N*-cyanomethyl-*o*-nitroanilines, and hence possessed the nitrile functionality at C-2. Such products were invaluable in attempts to build a structure-activity relationship for these benzimidazole nucleosides' action against a number of selected viruses.

*N*-cyanomethyl-4-fluoro-2-nitroaniline (148) was cyclised to 2-cyano-5-fluorobenzimidazole 3-oxide (149) by heating under reflux in ethanol with potassium carbonate. Subsequent reaction of (149) with 3-bromopropan-1-ol (104) yielded the desired product (150) in good yield. Previous workers had found difficulty in such reactions using sodium ethoxide as the base. In their attempted *O*-alkylations it had been found that such strongly nucleophilic bases not only brought about deprotonation of the *N*-oxide but, also attacked the electrophilic centre of the nitrile to furnish imidate esters. The  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra of compounds (149) and (150) were both very complex due to the  $^1\text{H}$ - $^{19}\text{F}$  and  $^{13}\text{C}$ - $^{19}\text{F}$  couplings.



Two other benzimidazole *N*-oxides were available for *O*-alkylation. Both 2-cyano-5-methyl- (151) and 2-cyano-5-methoxybenzimidazole 3-oxide (152) were reacted with 3-bromopropan-1-ol (104) to yield their corresponding hydroxypropoxy derivatives (153) and (154) respectively. Unfortunately, (154) could not be isolated in pure form to enable its testing against HSV1. Although peaks of a chemical shift corresponding to that of the desired product were evident, only a very small yield of the crude material was isolated. This was presumed to be due to the electron-donating nature of the methoxy group upsetting the electronic nature of the *N*-oxide and reducing the acidity of the N-1 proton.



The antiviral testing of the above compound (153) and those previously mentioned in this chapter will be discussed in chapter 5. The activity of a number of a selected compounds will also be discussed regarding their activity against a more varied panel of viruses.

## **Chapter 4**

### **Analogues with additional functionality**

#### **4.1 Introduction**

The myriad of antiviral agents discussed in chapter 1 illustrates that nucleoside analogues may have a host of sugars or acyclic moieties attached to the base, other than the natural sugars, ribose and 2-deoxyribose. Ara-C (12) and Ara-A (13) (chapter 1, p.5) have the base attached to an arabinose sugar. In AZT (17) the thymine is attached to a dideoxy sugar in which both 2'- and 3'-hydroxyl groups are missing, the 3'-hydroxyl being replaced by azido; other such dideoxy nucleosides include ddC (15) and ddI (16) (chapter 1, p7). Ganciclovir (23) (chapter 1, p.12) and a number of the SmithKline Beecham compounds (chapter 1, p13) synthesised on the way to famvir (29) possess a novel dihydroxy side-chain in which C-2' of the "sugar" is missing; in acyclovir (21) (chapter 1, p11) both C-2' and C-3' (i.e. the entire 'bottom half' of the sugar) have been removed.

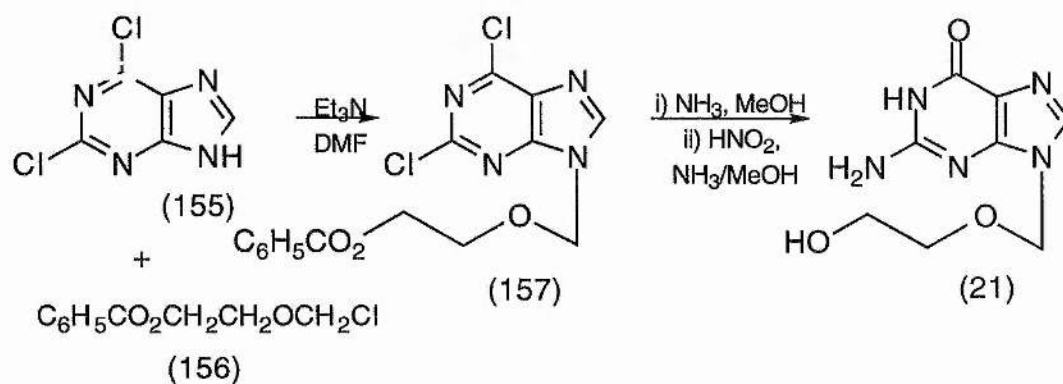
In all of the alkylations of benzimidazole *N*-oxides discussed previously in the present work, the alkylating agent has either been ethyl iodide, 3-bromopropan-1-ol, or a hydroxyl-protected 3-bromopropan-1-ol. The use of 3-bromopropan-1-ol introduces an acyclic portion to the resultant nucleoside analogue which is equal in "length" to that of acyclovir (i.e. the terminal OH is separated from the heterocycle by a chain containing the same number of atoms), but it produces a compound with an N-O-C rather than an N-C-O linkage between the base and the "sugar".

In the synthesis of those benzimidazole nucleosides previously discussed, all the benzimidazole *N*-oxides were synthesised prior to the *O*-alkylation step which yielded (in most cases) the desired product. It was thought worthwhile at this stage, however, to consider how

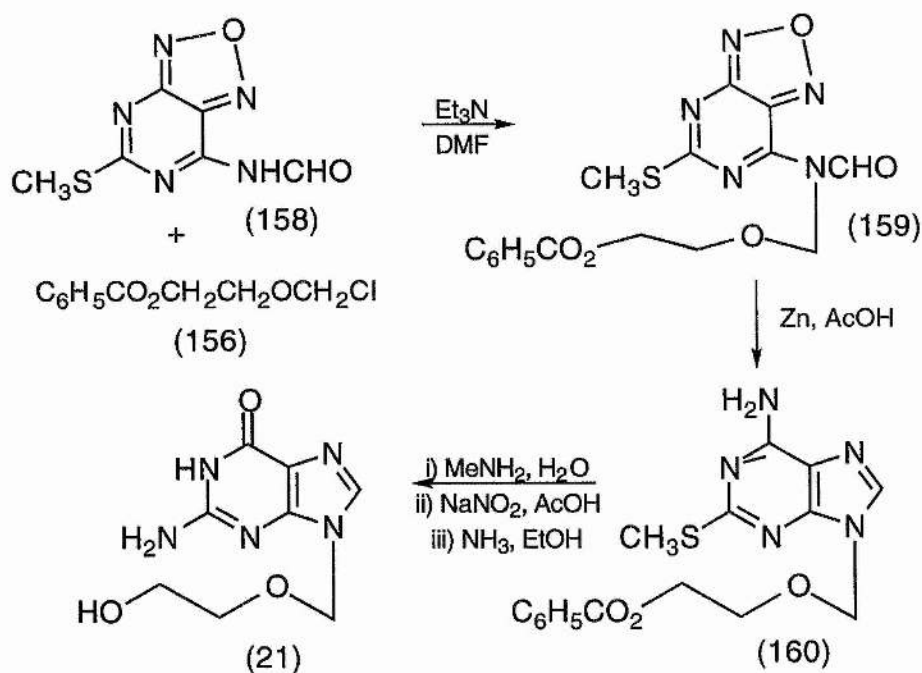
acyclovir, and ganciclovir and famvir with their more complex acyclic groups, were synthesised, and how such syntheses might be applicable, or adaptable, to the synthesis of *N*-alkoxybenzimidazoles with more complex alkoxy groups.

#### 4.2 Synthesis of antiviral nucleoside analogues with more complex side-chains

In the initial reports<sup>29</sup> about the activity of acyclovir (21), the synthesis was carried out via the *N*-alkylation of a suitably substituted purine (155) with the protected acyclic group (156), followed by the relevant functional group transformations and deprotection steps from (157) to the product, acyclovir (21). Through this general synthesis, other nucleoside analogues could also be produced. A subsequent unambiguous synthesis was reported by Kelley and Schaeffer<sup>102</sup> in which 7-formamido-5-methylthio-[1,2,5]-oxadiazolo-[3,4-*d*]-pyrimidine (158) is *N*-alkylated (at the eventual N-9 of the purine) with (156) prior to the formation of the imidazole ring of the final product (21): hence ring closure, deprotection of the side-chain, and formation of the correct functionality are carried out with the N(9) – C(1') bond intact.





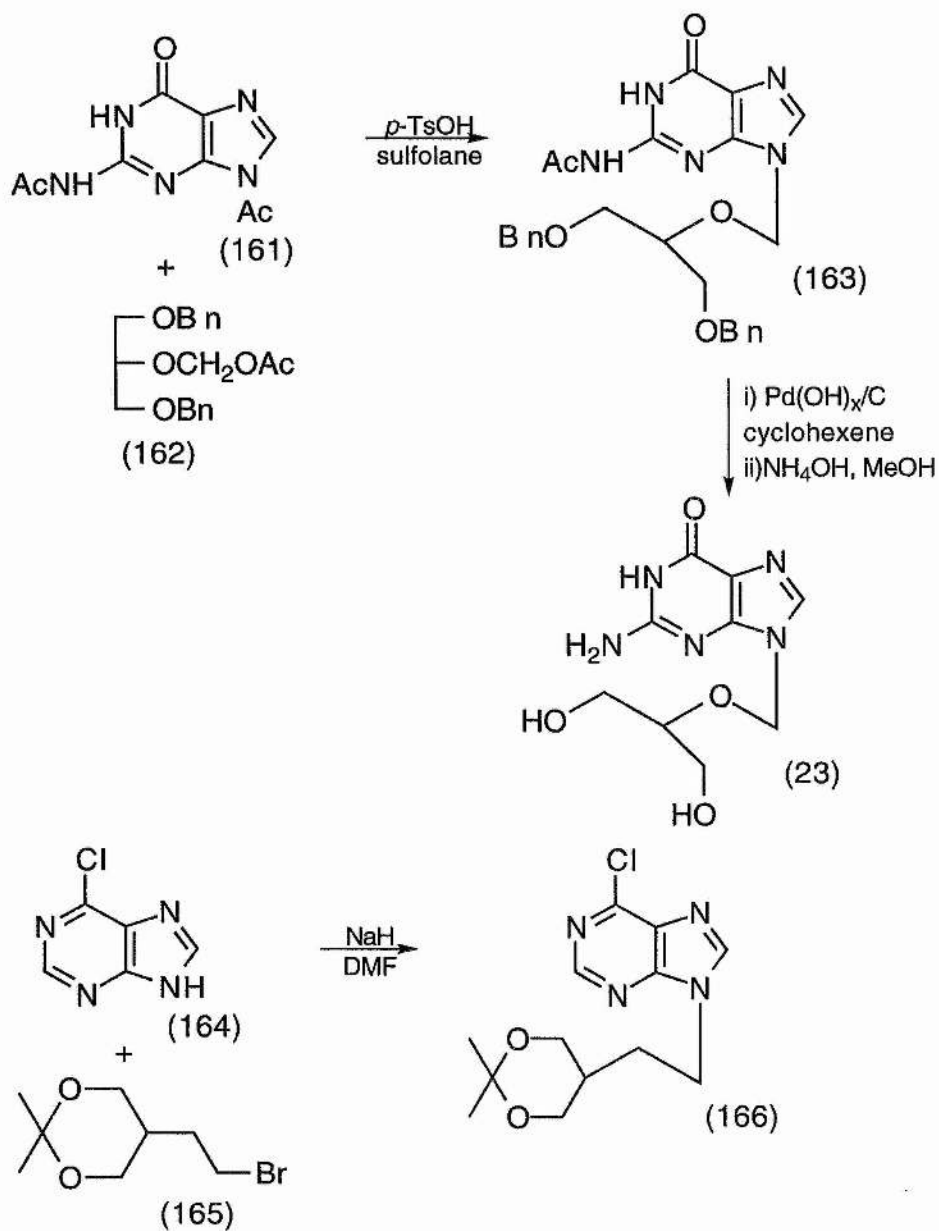


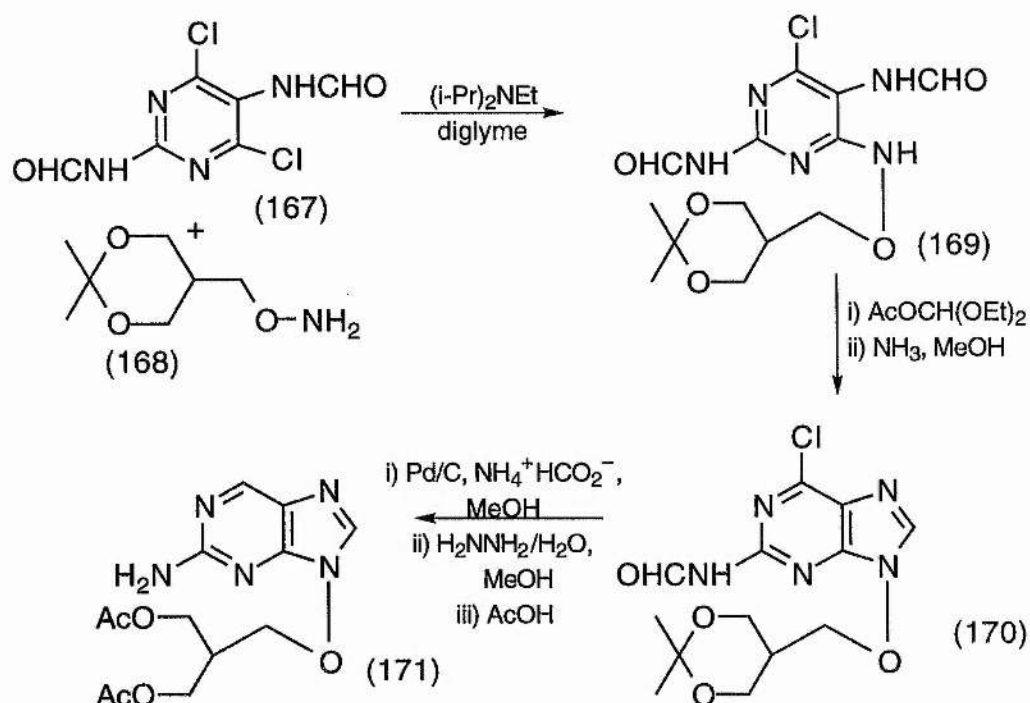
9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine, DHPG, or ganciclovir (23) may be synthesised by a procedure similar to the acyclovir (21) synthesis above. An analogue of the guanine base (161) is simply alkylated using the protected acyclic group (162) followed by simple deprotection steps to yield the desired product (23)<sup>34</sup>.

The phosphonate isosteres of both acyclovir<sup>103</sup> and DHPG<sup>104</sup> were also synthesised by the alkylation of suitable purines with protected alkylating agents.

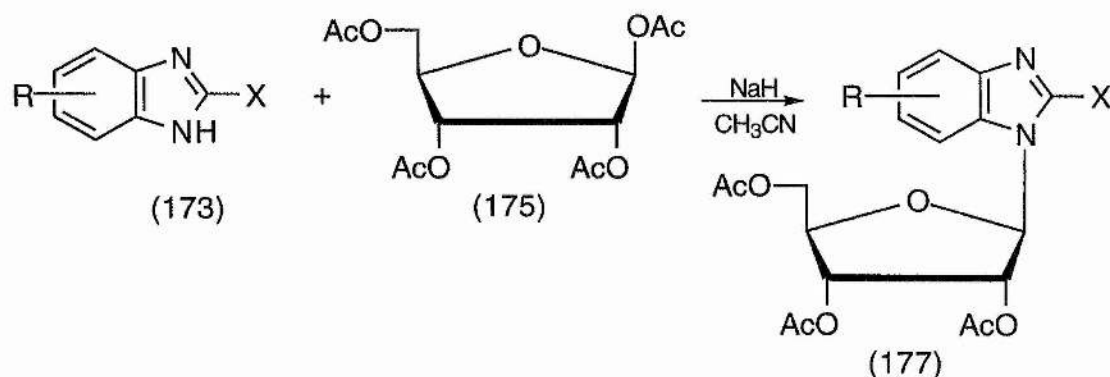
The work of Harnden, Jarvest *et al.*<sup>41-46</sup> at SmithKline Beecham's laboratories generally used both approaches to the final nucleoside analogue: both alkylation of the purine (164) with a protected acyclic moiety (165) and the alkylation of a functionalised pyrimidine (167) before closure of the imidazole ring (169). Where the final product possessed a carbocyclic side-chain (i.e. no "ribose" oxygen was present) (166), the alkylation was carried out on the preformed purine (164). In cases where the final product (171) contained an N-O-C linkage between the base and the "sugar", a suitably functionalised pyrimidine (167) was alkylated before the closure of the imidazole ring. The latter nucleoside analogues are of

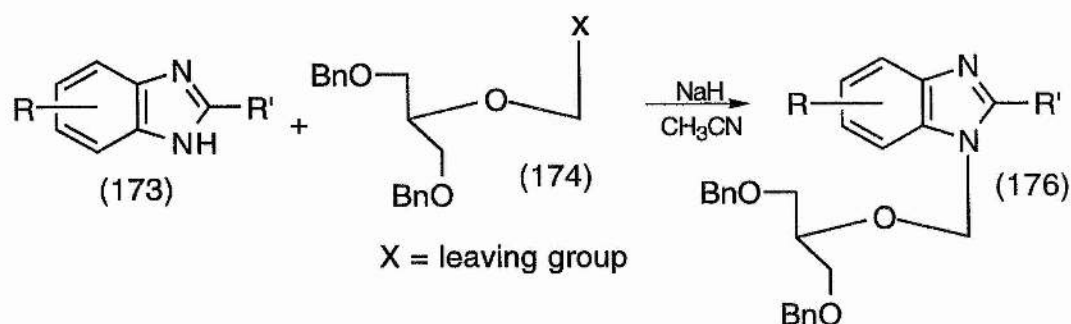
particular interest to the present study, as the benzimidazole nucleosides synthesised previously (chapters 2 and 3) contain an N-O-C linkage, and the more complex side-chains used in the syntheses of the commercial products may also be utilised in this work.



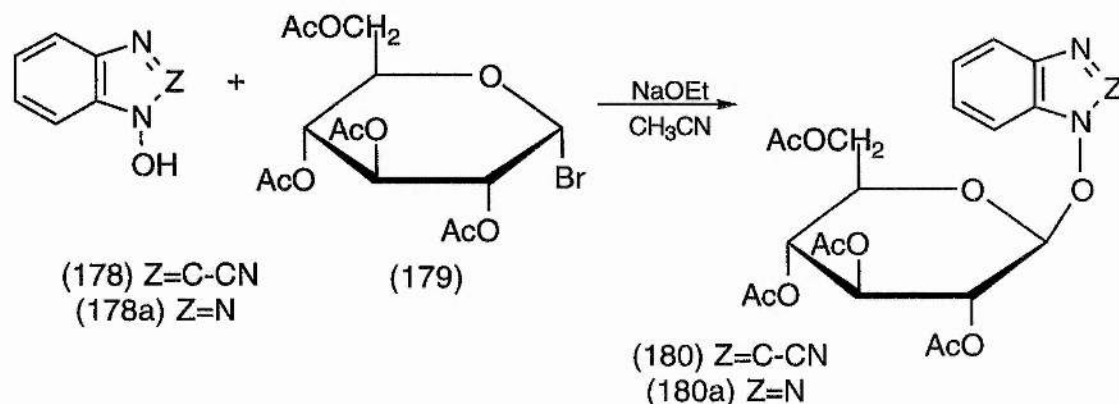


The benzimidazole nucleosides TCRB (38) and BDCRB (39) (chapter 3, p50) produced by Townsend *et al.* <sup>53,54,96</sup> were synthesised from suitably protected ribose sugars (175) and the desired benzimidazole (173) in a simple alkylation step followed by deprotection. Where an acyclic alkyl group was required (174), the heterocycle (173) was again directly coupled with the suitably protected alkyl moiety, as was the case for both acyclovir (21) and ganciclovir (23).





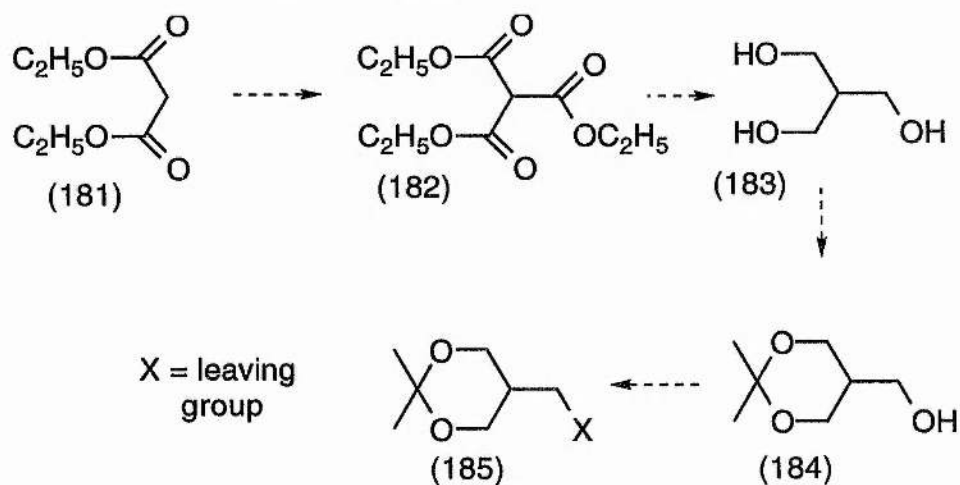
Benzimidazole and benzotriazole *N*-oxides (178) and (178a) had also been alkylated by Grochowksi and Falent-Kwastowa<sup>79</sup> with suitably protected sugars (179) to produce nucleosides (180 and 180a) with an N-O-C linkage between the base and the sugar. In contrast to the work of Harnden and Jarvest<sup>41-46</sup>, these alkylations were carried out on preformed benzimidazoles and benzotriazoles.



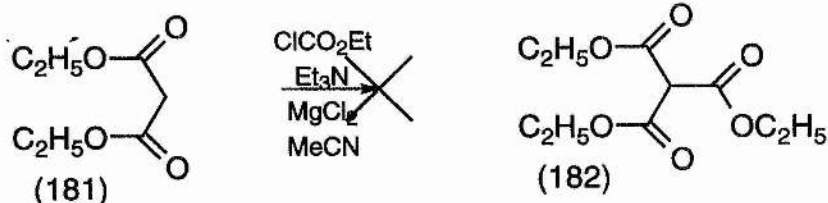
### 4.3 Synthesis of a more complex alkylating agent

It seems logical to assume that the production of a more complex alkylating agent like that used by Harnden *et al.*<sup>41-46</sup> should pose no problem in the majority of cases, and in this chapter approaches to more complex side-chains will be discussed. In order to produce a dihydroxy side-chain similar to those incorporated in ganciclovir (23) and famvir (29) the work

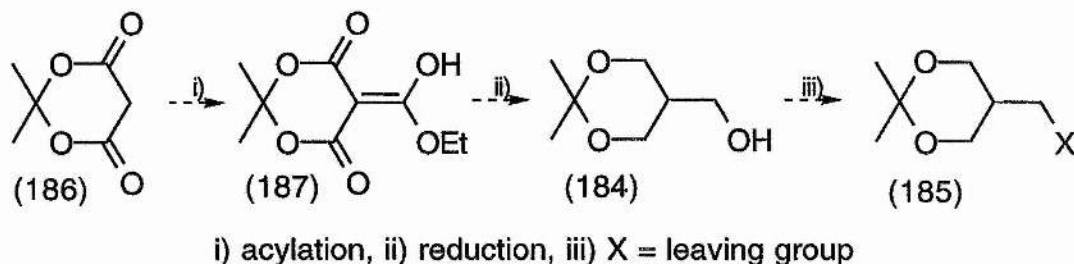
of Harnden and Jarvest<sup>42</sup> was modified to produce the proposed synthetic route shown below. To facilitate the formation of an alkyl substituent of the correct "length", with the correct N-O-C linkage, the alkylating agent (185) was required to contain one carbon less than that used in the SmithKline Beecham synthesis; hence diethyl malonate (181) was reacted with ethyl chloroformate rather than ethyl chloroacetate.



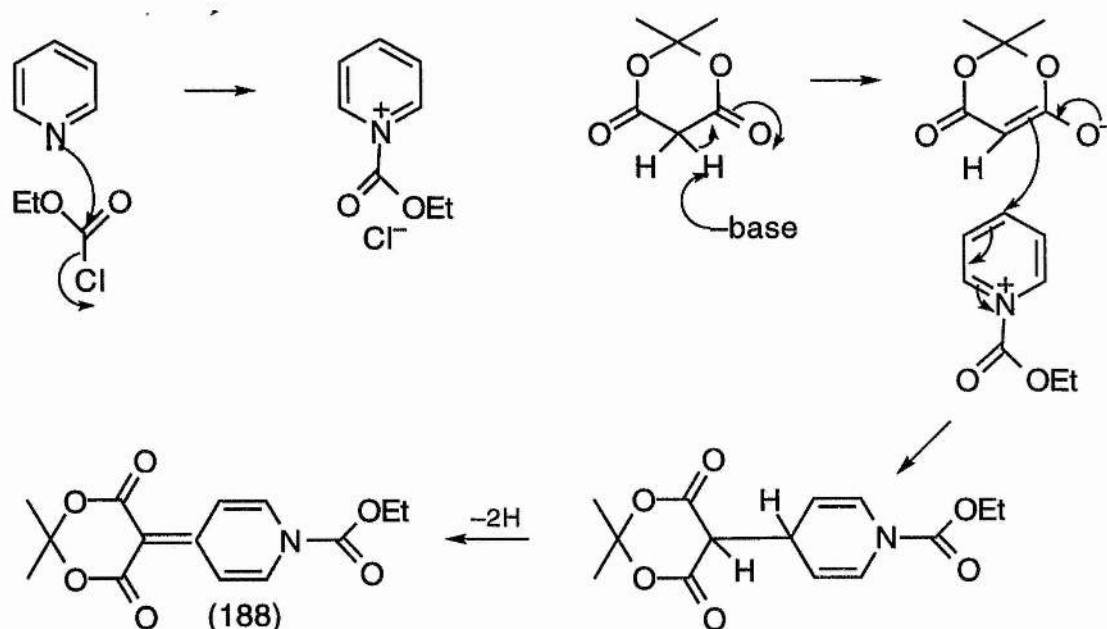
The acylation of diethyl malonate (181) was attempted under the conditions of Rathke and Cowan<sup>105</sup>. Two equivalents of base (triethylamine) were used, in order to prevent reaction of the enolate with the product; the latter, being a stronger acid than diethyl malonate, would be expected to reprotonate the malonate if only one equivalent of base were used, and thus prevent the desired reaction proceeding beyond 50% completion. According to Rathke and Cowan<sup>105</sup>, magnesium chloride was present in order to enhance the acidity of the malonate protons through complexation, so as to enable the use of a weaker base which would not react destructively with the chloroformate acylating agent. In any event, the reaction was unsuccessful, producing only unchanged diethyl malonate (181) and a high-melting yellow solid of as yet unknown constitution.



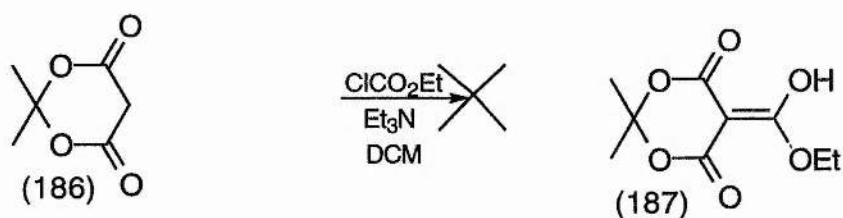
The failure of the above reaction was deemed unimportant, as a second route to the required protected diol had been devised, beginning from the well-documented Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione, or isopropylidene malonate) (186). The route contained one step less than the SmithKline Beecham-based route<sup>42</sup> and appeared simple to carry out. Acylation of Meldrum's acid by ethyl chloroformate, followed by reduction to the protected triol (184) and bromination, would yield the desired alkylating agent (185). This chemistry had also been previously reported elsewhere<sup>106</sup>.



Once again, the reaction with ethyl chloroformate was not as straightforward as expected. Under the conditions employed<sup>107</sup>, using pyridine as the base, an unexpected product containing aromatic protons in the <sup>1</sup>H nmr spectrum was isolated. This product (188) was presumed to be an adduct formed between one equivalent each of the pyridine, Meldrum's acid and ethyl chloroformate. The mechanism proposed for the formation of the isolated product is shown below; a search of the literature revealed that the product was in fact a known compound<sup>108</sup>.

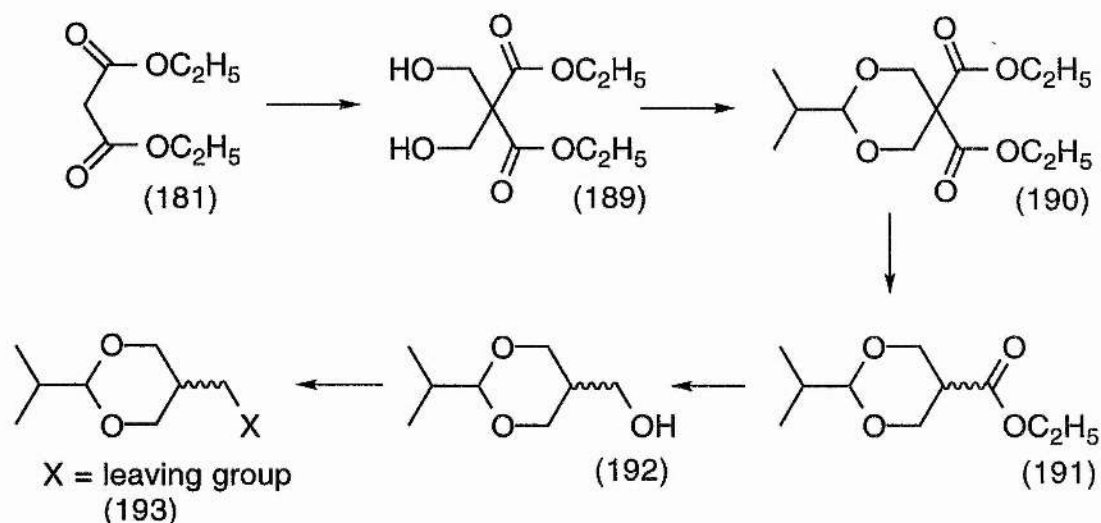


The reaction between ethyl chloroformate and Meldrum's acid (186) was repeated using triethylamine as the base [in order to prevent the formation of complex adducts such as (185)], but no reaction apparently occurred.

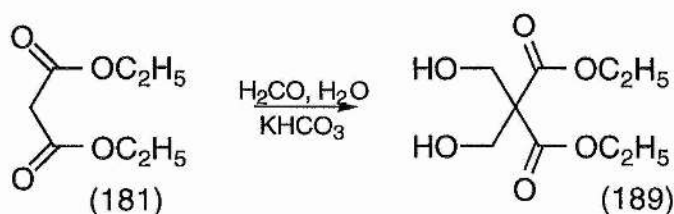


The previous two proposed synthetic routes having "fallen at the first hurdle", a third approach was tried. A complete synthesis of 2-hydroxymethylpropane-1,3-diol had been found<sup>109</sup>, and could be utilised with modification required only for the final step. The synthesis involved the reaction of diethyl malonate (181) with formaldehyde and protection of

the diol (189) formed as the dioxane derivative (190). Decarbethoxylation followed by reduction then yields the required protected triol (192), with a suitable leaving group (193) reaction with a benzimidazole *N*-oxide may be possible.

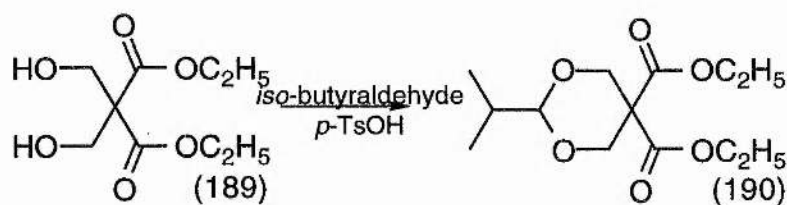


Diethyl malonate (181) was reacted with an aqueous solution of formaldehyde in the presence of potassium hydrogen carbonate<sup>110</sup>; the desired product was isolated but proved very difficult to purify. The low-melting solid was eventually recrystallised by stirring the crude product at very low pressure, to allow the evaporation of trapped residues of solvent, starting materials and water. Thereafter, in all repetitions of this step seeding with pure crystals was employed to produce the desired solid product.

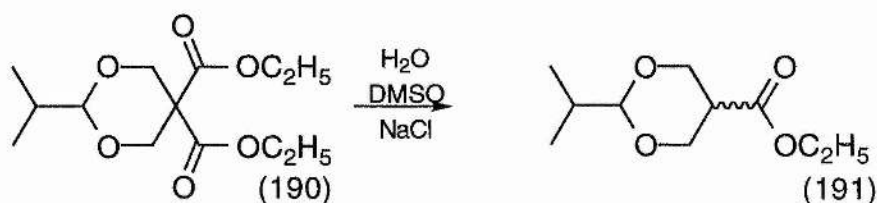




Protection of the diol (189) was undertaken using isobutyraldehyde with a catalytic amount of *p*-toluenesulphonic acid. Reaction in the initial solvent, petroleum (bp 40-60°C)<sup>111</sup>, yielded only a very small amount of the product; however, the use of toluene as solvent delivered the product in a much more respectable yield.



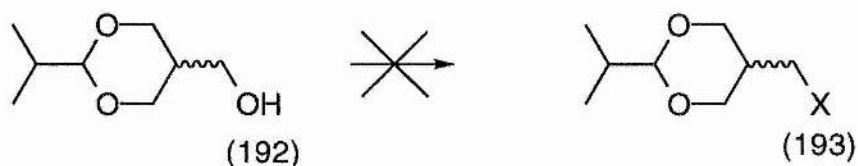
The diester (190) then underwent decarboxylation according to the method of Krapcho *et al.*,<sup>109,112</sup> to yield a mixture of diastereomeric monoesters. Sodium chloride is required to promote the decarboxylation. The <sup>1</sup>H and <sup>13</sup>C nmr spectra both clearly show the mixture of diastereomers, but the proportion of each cannot be determined from the integration, since the resolution is not good enough. The isopropyl group is expected always to assume the more stable equatorial position, and hence both axial and equatorial ethoxycarbonyl groups will be evident. Presumably the more thermodynamically stable stereoisomer will be the *trans* isomer, in which one conformation will have both the isopropyl and ethoxycarbonyl groups in equatorial positions.



The mixture of *cis*- and *trans*- 5-ethoxycarbonyl-2-isopropyl-1,3-dioxanes was reduced to the corresponding mixture of *cis*- and *trans*- 5-hydroxymethyl-2-isopropyl-1,3-dioxanes using lithium aluminium hydride in THF<sup>111</sup>. The product was purified by distillation: a second distillation was required to remove all traces of the starting material.



The final stage of the synthesis was the conversion of the hydroxyl of (192) to a suitable leaving group. It was thought that the most simple method to provide a good leaving group was tosylation. This process would introduce a labile tosyl group for the reaction of (193) with a benzimidazole *N*-oxide.

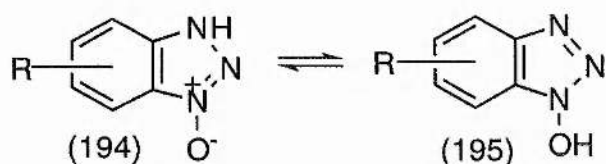


The reaction of (192) with tosyl chloride did not succeed under a variety of conditions, even after the recrystallisation of tosyl chloride to remove traces of *p*-toluenesulphonic acid. The addition of 4-dimethylaminopyridine (DMAP) did nothing to promote the reaction<sup>113</sup>. In each case the <sup>1</sup>H nmr showed a small trace of the desired product but tosyl chloride continued to precipitate out of the isolated oily material; it was proposed that the tosyl chloride had either

dissolved in the starting material (192) and was crystallising out of solution or that the reaction had succeeded but was reversible.

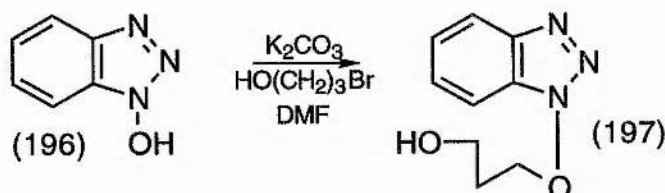
#### 4.4 Alkylation of functionalised benzotriazole *N*-oxides

Benzotriazole *N*-oxides or 1-hydroxybenzotriazoles have long been used in the synthesis of peptides in order to prevent the racemisation at stereogenic centres during peptide coupling<sup>114</sup>. Benzotriazole *N*-oxides have a very similar structure to benzimidazole *N*-oxides and may also be similarly functionalised on the benzene ring [like benzimidazole *N*-oxides, benzotriazole *N*-oxides (194) are tautomeric with 1-hydroxybenzotriazoles (195)]. The reaction of benzotriazoles with acyclic alkylating agents had been previously investigated<sup>115</sup> and benzotriazole *N*-oxides have also been alkylated on the oxygen, but in this case with protected sugars<sup>79</sup>. The resultant compounds would presumably possess similar functionality to the previously synthesised benzimidazole nucleosides but bear different functionality at the 2-position; a nitrogen atom would replace the carbon or functionalised carbon. This would prove very interesting in an antiviral assay as Townsend *et al.*<sup>54</sup> had deemed a chlorine or bromine a necessity at C-2 for the maintenance of the activity and absence of toxicity.



In the first instance commercially available 1-hydroxybenzotriazole (196) was reacted with 3-bromopropan-1-ol (104) in DMF using potassium carbonate as the base. The reaction yielded the desired product (197) which was isolated and purified by distillation. On standing

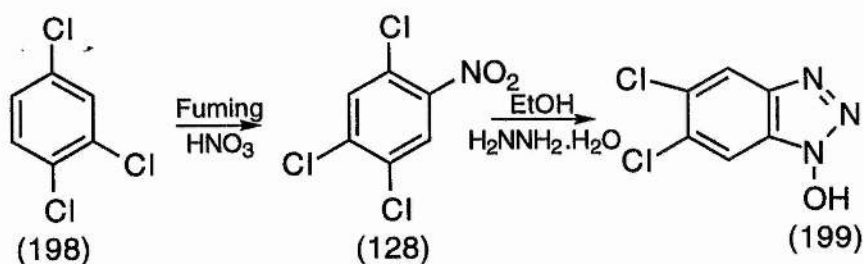
in the atmosphere, the clear oily product darkened in colour but the  $^1\text{H}$  nmr showed no change to have occurred in the product and further distillation at reduced pressure removed the discolouration.



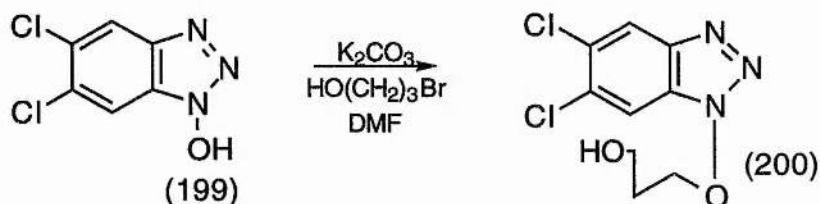
As the synthesis of (197) had proved so simple it was decided to synthesise the 5,6-dichlorobenzotriazole *N*-oxide to enable a direct comparison of the effects at the 2-position upon 5,6-dichloro-substituted nucleoside analogues [cf. (136) and (137), chapter 3, p56].

Substituted 1-hydroxybenzotriazoles may be synthesised via the heating of a suitably substituted *o*-halogenonitrobenzene with hydrazine hydrate in ethanol under reflux<sup>116</sup>. Nucleophilic substitution of the halogen occurs, followed by spontaneous cyclisation to the benzotriazole *N*-oxide. This involves attack of the lone pair of the second nitrogen at the electrophilic centre of the *o*-nitro group.

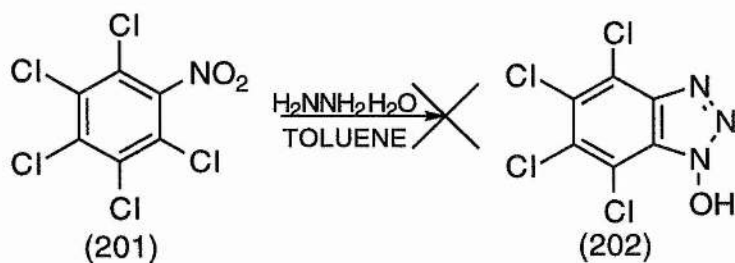
1,2,4-Trichlorobenzene (198) was nitrated with fuming nitric acid at room temperature to yield the desired 1,2,4-trichloro-5-nitrobenzene<sup>98,117</sup>. The trichloronitrobenzene (128) was then heated under reflux in ethanol with hydrazine hydrate to yield directly 5,6-dichlorobenzotriazole 1-oxide (199)<sup>116</sup>.



The benzotriazole *N*-oxide (199) could then be alkylated in the normal manner with 3-bromopropan-1-ol (104). The desired product (200) was isolated in good yield.



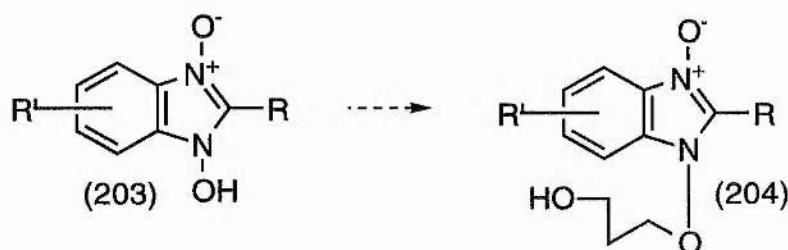
Several attempts were made to synthesis 4,5,6,7-tetrachlorobenzotriazole 1-oxide (202) from pentachloronitrobenzene (201). The solvent required, according to the literature method<sup>116</sup>, was toluene in order to maintain the lipophilic pentachloronitrobenzene in solution, but in each case the desired product was not isolated.



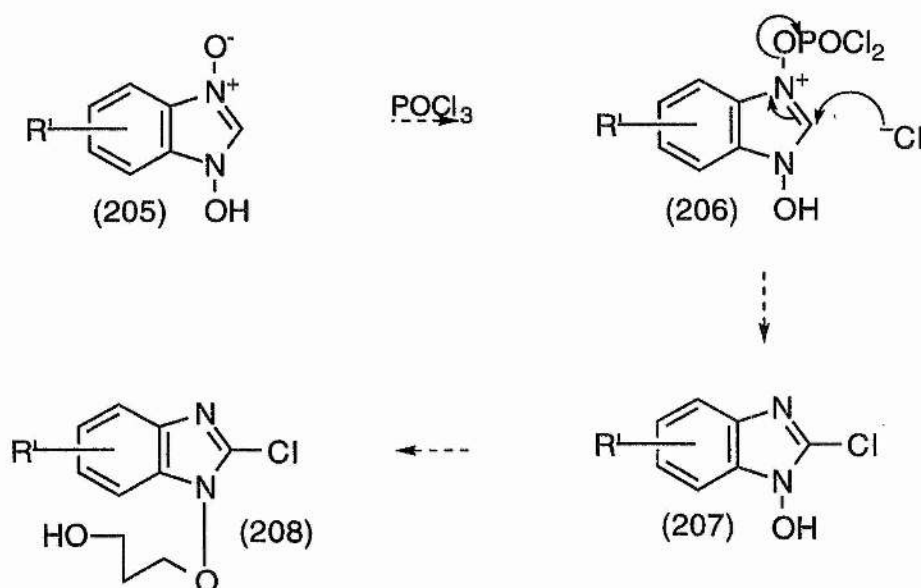
The compounds (197) and (200) were submitted for antiviral testing (chapter 5).

#### 4.5 Attempts to synthesise 1-hydroxybenzimidazole 3-oxides.

It was mentioned in chapter 1 that the *N*-oxides of several purines possessed biological activity. It was postulated that the synthesis of 1-hydroxybenzimidazole 3-oxides (203), like benzimidazole 3-oxides, could be alkylated on one of the oxygen atoms to furnish a nucleoside analogue (204) with an *N*-oxide at one nitrogen and an N-O-C linkage to the acyclic side chain at the other. Such compounds would have points of similarity with the purine *N*-oxides mentioned above (chapter 1, p20) and also with other nucleoside analogues.

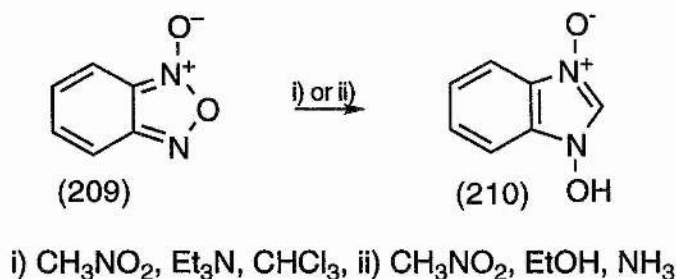


1-Hydroxybenzimidazole 3-oxides (205) may also be used for the introduction of substitution at C-2 (208), dictated as a necessity for activity by the results of Townsend *et al.*<sup>54</sup> (see below).



1-Hydroxybenzimidazole 3-oxides may be synthesised from benzofurazan *N*-oxides by reaction with a variety of carbanions. Such syntheses also yield quinoxaline-di-*N*-oxides<sup>118</sup>; the product furnished depends upon the carbanion used. It was reported<sup>119</sup> that the reaction of benzofurazan *N*-oxide with nitromethane would furnish 1-hydroxybenzimidazole 3-oxide, unsubstituted on the benzene ring.

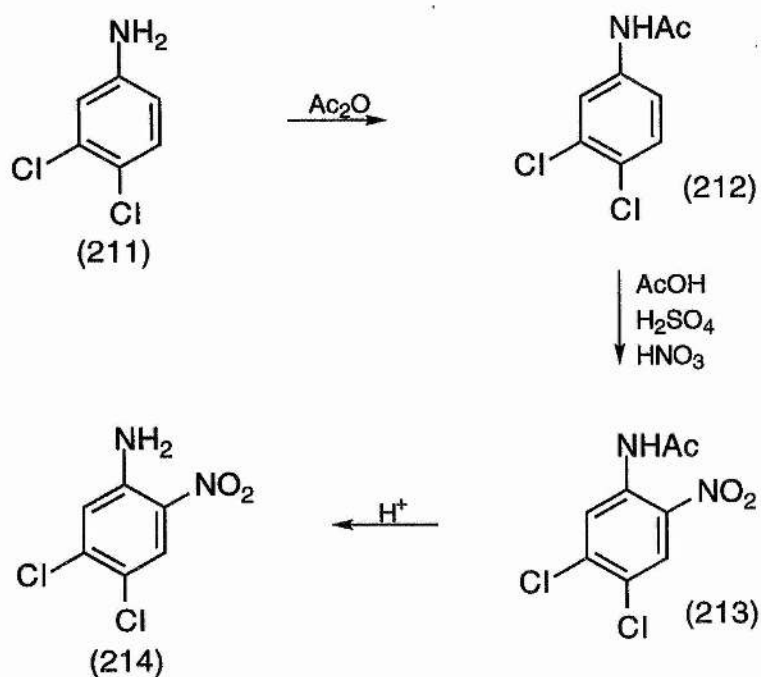
In the first instance benzofurazan *N*-oxide (209) was heated under reflux in chloroform with triethylamine and nitromethane using the conditions of Meth-Cohn *et al.*<sup>119</sup>, but no evidence of the desired product (210) was found. The reaction was repeated after recrystallisation of the benzofurazan *N*-oxide (209) and the drying of both the nitromethane and chloroform. This time the product was identified spectroscopically through <sup>1</sup>H nmr analysis of the crude reaction mixture, but the desired product could not be isolated in a usable amount.



The above reaction was then attempted using different conditions<sup>120</sup>, ammonia dissolved in ethanol as the base for the creation of the carbanion from nitromethane. Again the reaction was not a success, returning only the unreacted starting material.

In parallel with the above work, the synthesis of 5,6-dichlorobenzofurazan *N*-oxide was attempted to enable the eventual synthesis of 1-hydroxy-2,5,6-trichlorobenzimidazole 3-oxide. 3,4-Dichloroacetanilide (212) was synthesised from 3,4-dichloroaniline (211) by reaction with acetic anhydride<sup>121</sup>. The acetanilide (212) was nitrated under the conditions of

for the synthesis of the 5,6-dichlorobenzofurazan *N*-oxide. 4,5-Dichloro-2-nitroaniline (214) was obtained from the *o*-nitroacetanilide (213) by reflux in acid.



As attempts to synthesise the 1-hydroxybenzimidazole 3-oxide had failed, the synthesis of 5,6-dichlorobenzofurazan 3-oxide was pursued no further.



## **Chapter 5**

### **Antiviral assays**

#### **5.1 Introduction**

The activities of acyclovir (21), ganciclovir (23), famvir (29) and its analogues, and the benzimidazole nucleosides (38 and 39) produced by Townsend *et al.* against a variety of viruses have already been covered in chapter 1. In the following three chapters, the syntheses of various nucleoside analogues have been described. The nucleoside analogues produced possess functionality similar to that of the commercial products and of those others of proven antiviral activity. In this chapter the testing of some of these new compounds against herpes simplex virus 1 (HSV1) will be described. An outline of the procedure is described first, and the results then discussed.

A number of the *N*-alkoxy-benzimidazoles and -benzotriazoles synthesised by the author, and some benzimidazole *N*-oxides arising from previous work in the group, have also been submitted to the Wellcome Research Laboratories (now part of Glaxo Wellcome) for testing against a panel of viruses. The results of these tests are given later in this chapter.

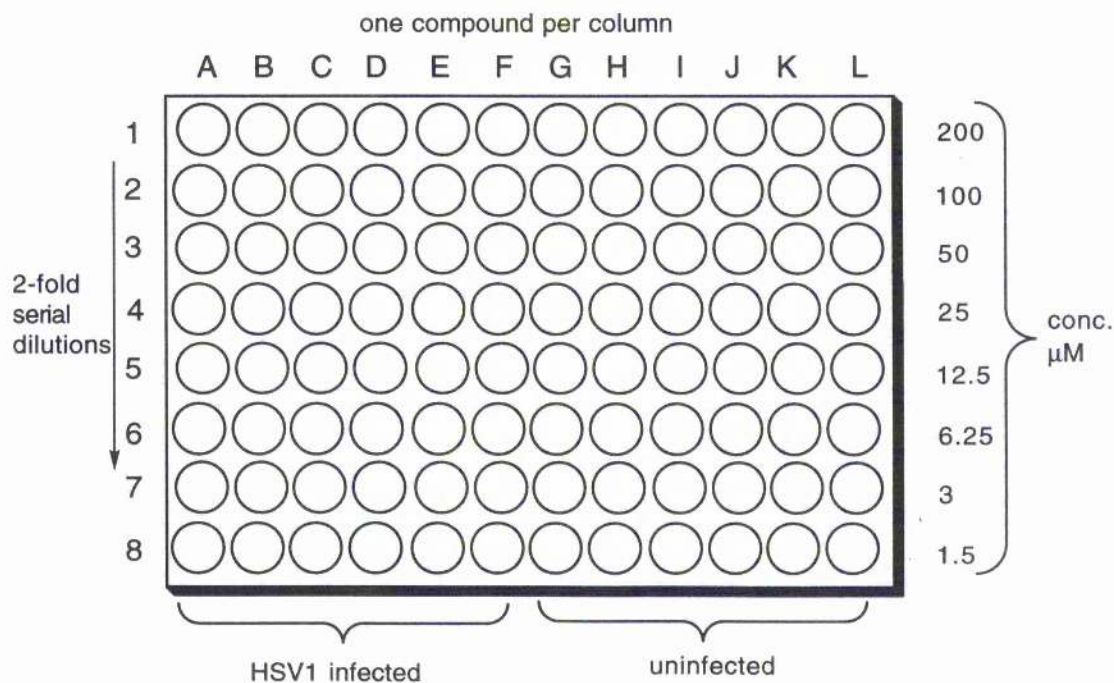
#### **5.2 The basic assay**

(A more detailed account of the experimental methods and the materials used is given in the experimental section of the thesis)

Vero cells were grown to a confluent monolayer in a 96-well microtitre plate over 48 hours.

The virus stock solution was diluted 1 in 100 000 with Glasgow-modified Eagle's medium and 10% calf serum (G-MEM/10% CS), and the solutions of the compounds for testing were also diluted to a maximum of *ca.* 200  $\mu$ M with cell medium (G-MEM).

In a 96-well microtitre plate, 150  $\mu$ l of the HSV1-infected cell medium was added to each well. To the top well of each column, a further 150  $\mu$ l of the compound in cell medium at 200  $\mu$ M was added, and two-fold serial dilutions made down the column. This was repeated for each compound (one compound per column). One column was tested with DMSO alone, at the same concentration used in the testing solutions, as a control.



In half of the assays, the plate was only infected with HSV1 in columns A-F. Columns G-L being left uninfected as a test for toxicity of the benzimidazole derivatives. In the other cases, two plates were set up; one infected with the virus and the other only containing the testing compounds, again as a toxicity test.

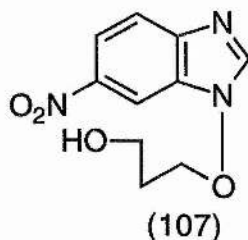
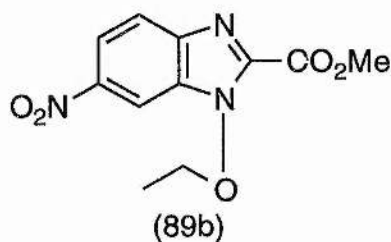
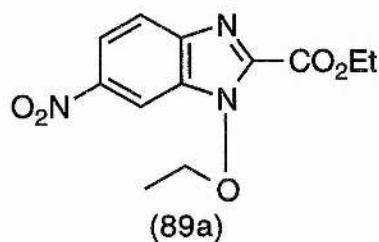
The contents of each 96-well microtitre plate were then transferred to the plates containing the cells after the removal of the old cell medium. 100  $\mu$ l of solution was placed in each well. The cells were then incubated at 37°C for up to 72 hours, with checking every 8-12 hours.

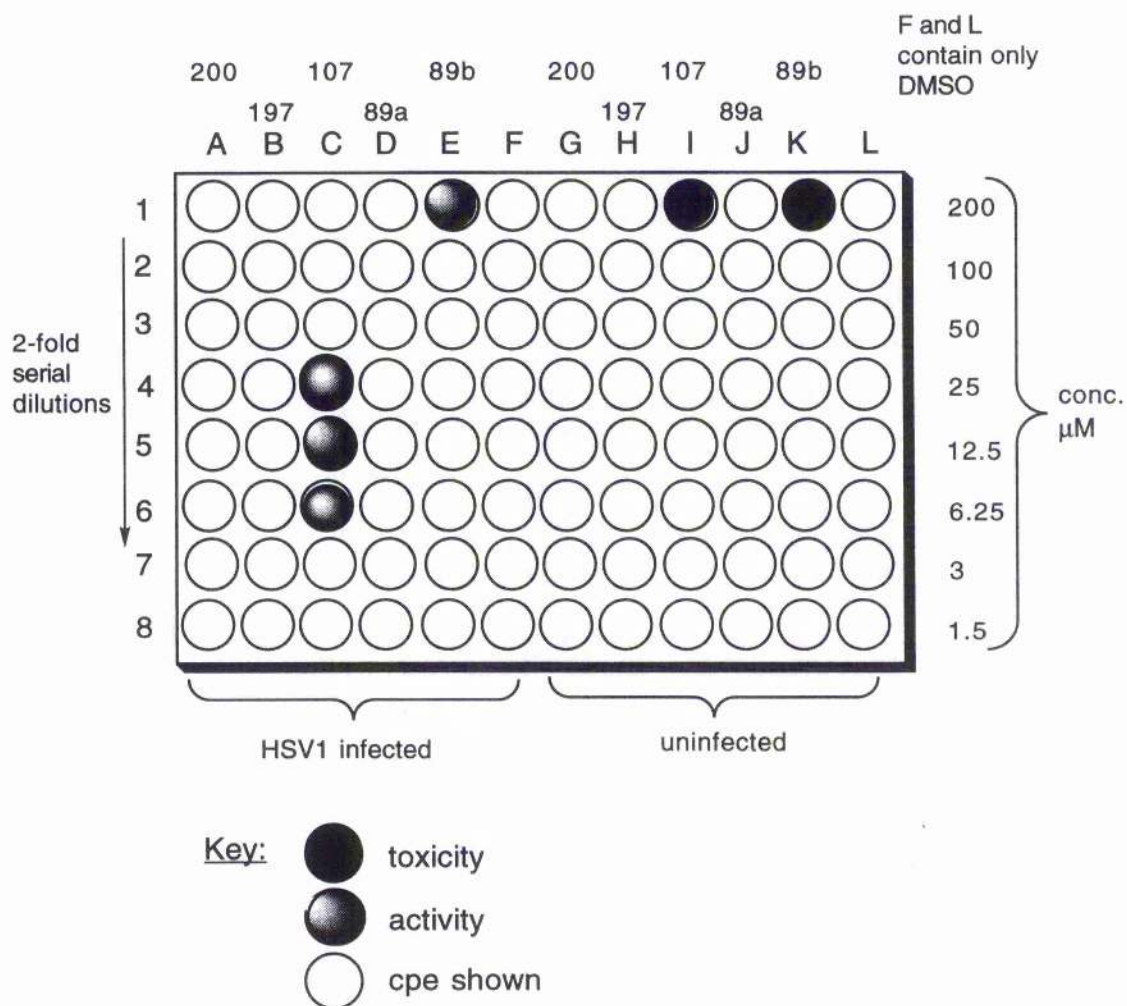
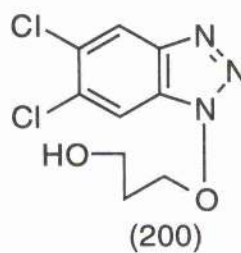
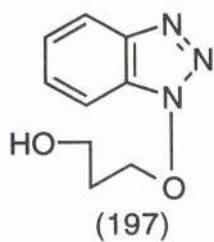
After analysis of the cells under the microscope, they were fixed with 2 % formalin and stained with crystal violet.

### 5.3 Assay 1

In assay 1 the general procedures previously outlined were followed except that the 96-well microtitre plate used had columns A-F infected with HSV1 and columns G-L were kept uninfected.

The compounds tested were as follows (continued overleaf):





In this assay the cells were incubated for 48 hours post-infection before fixing and staining.

In all but one case, the monolayer of cells showed considerable viral cpe at all concentrations of compound. In the uninfected monolayers, there was only one case of toxicity.

Compound (107), however, had intact monolayers at up to *ca.* 25-6  $\mu\text{M}$  concentrations. In the uninfected cells, however, at concentrations of *ca.* 200  $\mu\text{M}$  (the highest concentration of compound tested), there was evidence of toxicity.

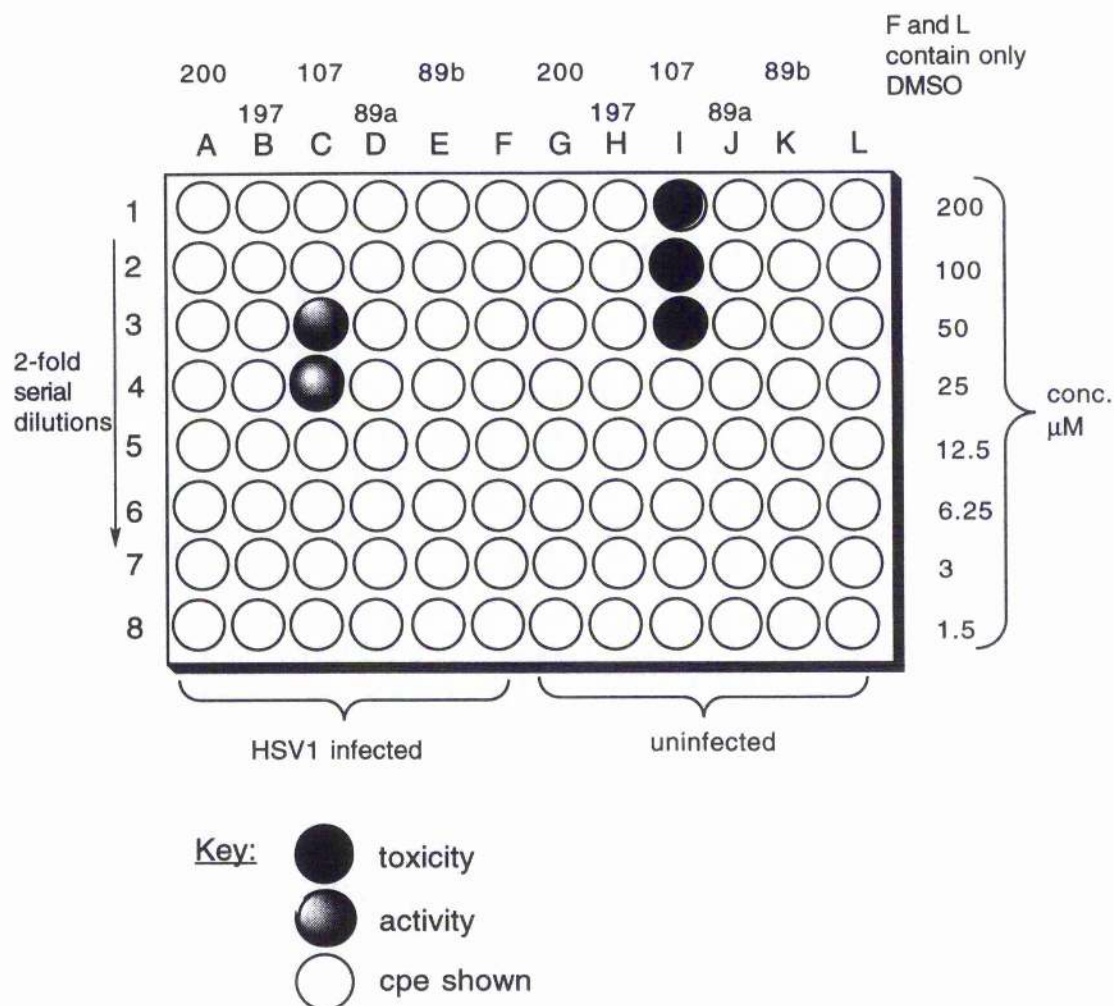
In the infected monolayer of cells containing compound (89b) at 200  $\mu\text{M}$  concentration there was slightly less fusion due to the cpe of the virus, but again in the uninfected cells there was evidence of toxicity at 200  $\mu\text{M}$ .

#### 5.4 Assay 2

Assay 2 was, strictly speaking, a repeat of assay 1. The microtitre plates were set up as for assay 1, but in triplicate using the same compounds. A repeat was necessary to ascertain if the test was reproducible and if a plausible activity was due to the compound being tested and not to accidental contamination. The plates were fixed and stained at three different periods post infection, this was to establish if the compounds were slowing the replication of the virus or stopping replication altogether.



## 5.4 Assay 2

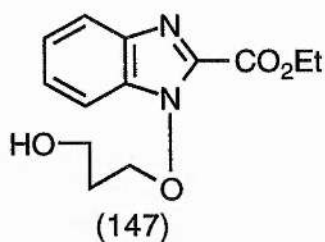
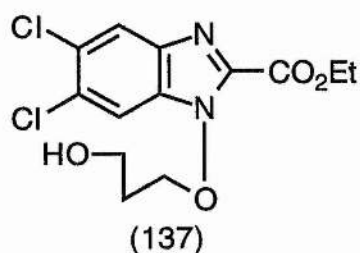
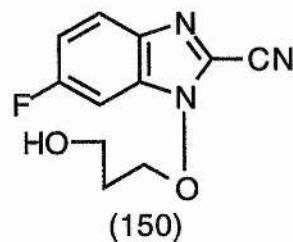
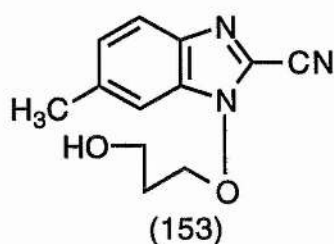
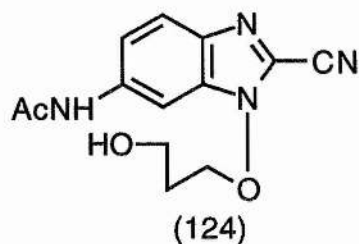
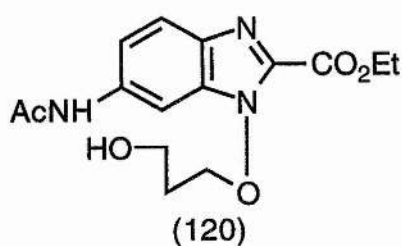
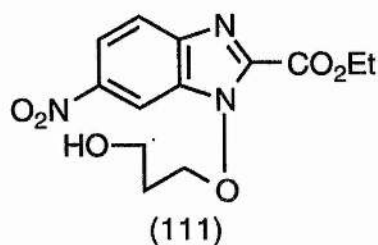
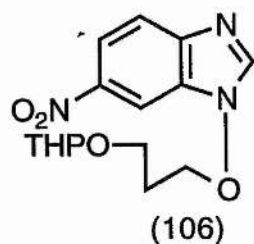


The monolayers of cells in the first microtitre plate were fixed and stained at 32 hours post infection and the other two plates at 56 and 80 hours. In each case the activity and toxicity results were very similar to those of assay 1. Only compound (107) showed any activity at 32 hours post-infection; the activity in this assay was at a lowest concentration of 25  $\mu\text{M}$ . In conjunction with this, evidence of toxicity was displayed at *ca.* 200  $\mu\text{M}$  in the uninfected monolayer. As time progressed, the concentration at which activity was displayed became higher, being between 25 and 50  $\mu\text{M}$  with 56 hours post infection, and at much higher concentrations at 80 hours post infection. In contrast, as time progressed, the toxic effects were observed at lower concentrations, 100-50  $\mu\text{M}$  at 80 hours post infection. Obviously the virus

was not prevented from replicating, and the compounds tested in this case were merely slowing down the progress of the virus. The slowing of the viral cycle was probably due to the benzimidazole nucleosides having a toxic effect on the cells. The virus, being only a parasitic mixture of proteins and fragments of nucleic acids requires a healthy cell with which to interact and replicate. The cell machinery which is 'hijacked' is required to be in perfect working order. Where a cell is exhibiting evidence of toxicity (e.g. due to the effects of a foreign compound) its biochemical machinery is not functioning correctly, and so the virus cannot replicate. Hence the virus cannot display its normal cpe in infected cells, and as a result an intact monolayer is seen. In the absence of virally uninfected cells which manifest this toxicity in a number of ways, the intact monolayer may be mistakenly interpreted as representing antiviral activity in infected cells.

### **5.5 Assay 3**

In this assay two microtitre plates were used, one was infected with HSV1 and the other left uninfected. Compound (107) was included in this assay as a marker for the levels of concentration at which viral progress was slowed over time and also at which level toxicity was detected. The compounds tested were as follows:

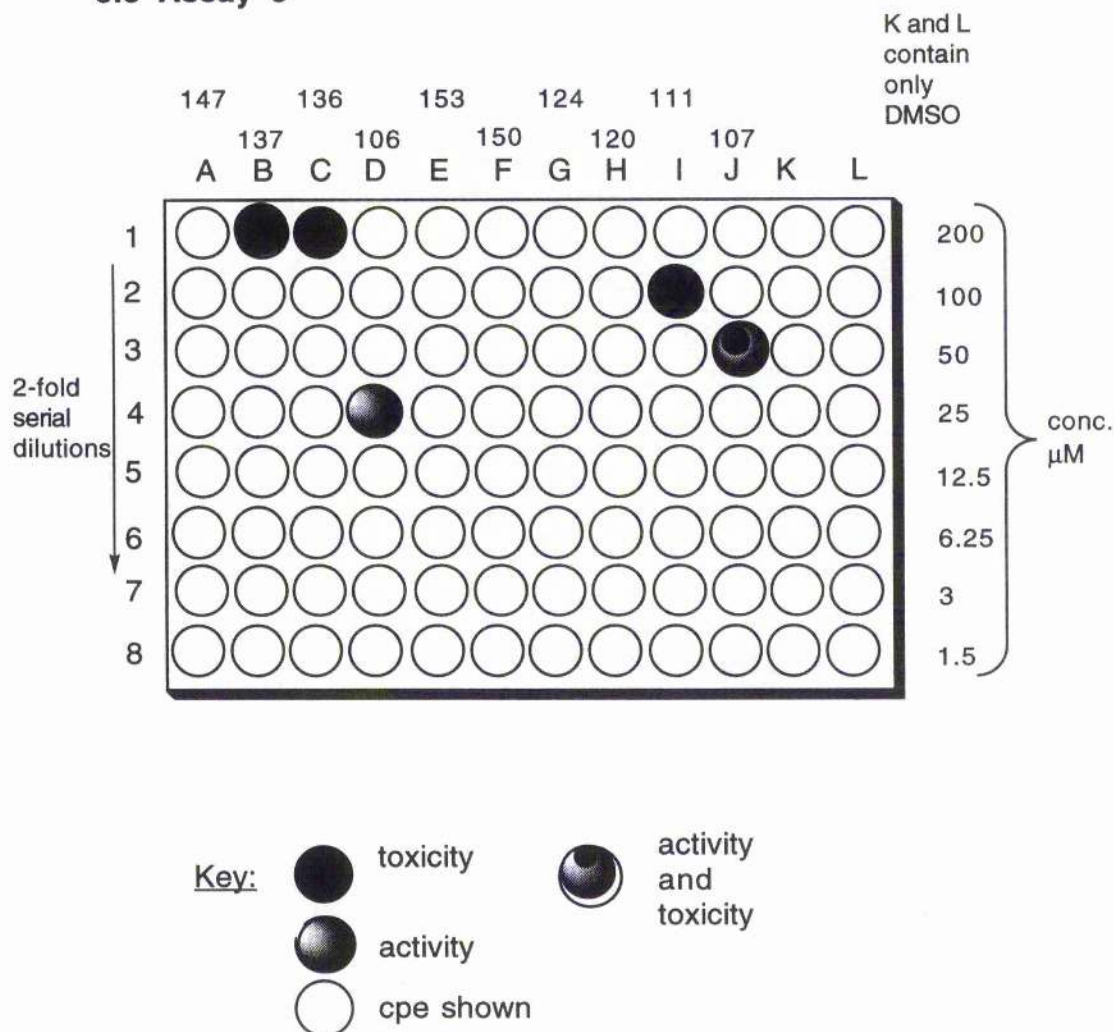


Of the compounds tested in this assay and fixed/stained after 48 hours, only one further compound seemed to exhibit any activity, but this was accompanied by toxicity at high concentration. Compound (106) the THP-protected ether of compound (107), showed activity at slightly lower concentration (25  $\mu$ M) than compound (107) itself (50  $\mu$ M). The uninfected plate showed that this compound also displayed toxicity at this low concentration. Compound (111),



an analogue of (107) with ester functionality at C-2, did not display activity- cpe was shown at all concentrations- but it did show evidence of toxicity at *ca.* 100 $\mu$ M.

### 5.5 Assay 3

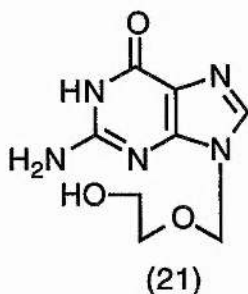


Toxicity was also displayed with compounds (139) and (136) at *ca.* 200 $\mu$ M, but in the corresponding infected monolayers viral cpe was displayed at all concentrations.

After only 24 hours of incubation, all of the compounds displayed marked cpe of the virus at lower concentrations. The higher concentrations of compound showed no cpe but this was presumably due to toxicity of a lesser degree.

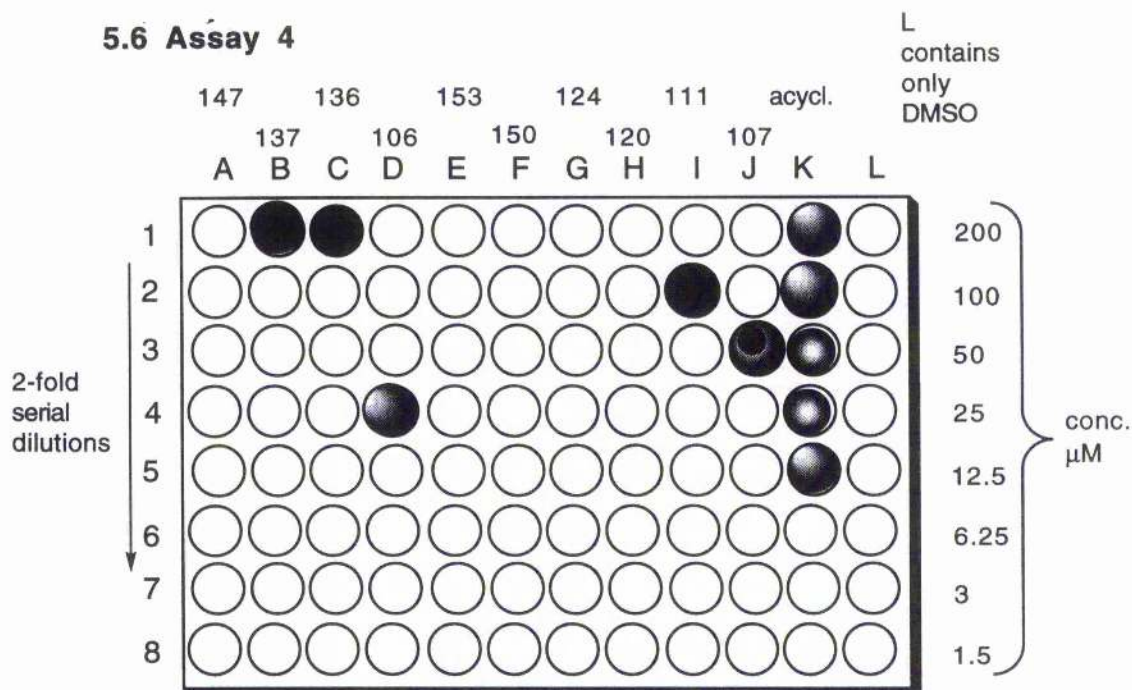
### 5.6 Assay 4

This assay involved an almost identical repeat of assay 3, with two plates being set up: one uninfected and the other infected. In the spare column, acyclovir (supplied by ICN Pharmaceuticals Ltd.) was placed at a maximum dilution of *ca.* 200  $\mu$ M in fresh DMSO.



The acyclovir was used as a positive control in the assay, to establish if the concentration of virus being used was controllable with a known, highly effective and commercially available therapy. Many variables are present in this type of assay, and the use of a known product as a control allows a more valid comparison of the activity and toxicity of the compounds tested with other, known compounds. A repeat of assay 3 was also required in any case to confirm the reproducibility of the results.

## 5.6 Assay 4



Key:

- toxicity
- activity
- cpe shown
- activity and toxicity

The results obtained in this assay proved to be almost identical to those of assay 3. Acyclovir proved to be active at concentrations as low as 12.5  $\mu\text{M}$  with no toxicity displayed at any level.

The tests carried out on these newly synthesised benzimidazole nucleoside analogues have shown them to possess an activity against HSV1 which is linked closely to the toxic effects which these compounds exert upon the uninfected cells. In the tests carried out in parallel with acyclovir, the activity of the market leader was shown to be excellent, with no trace of toxicity.

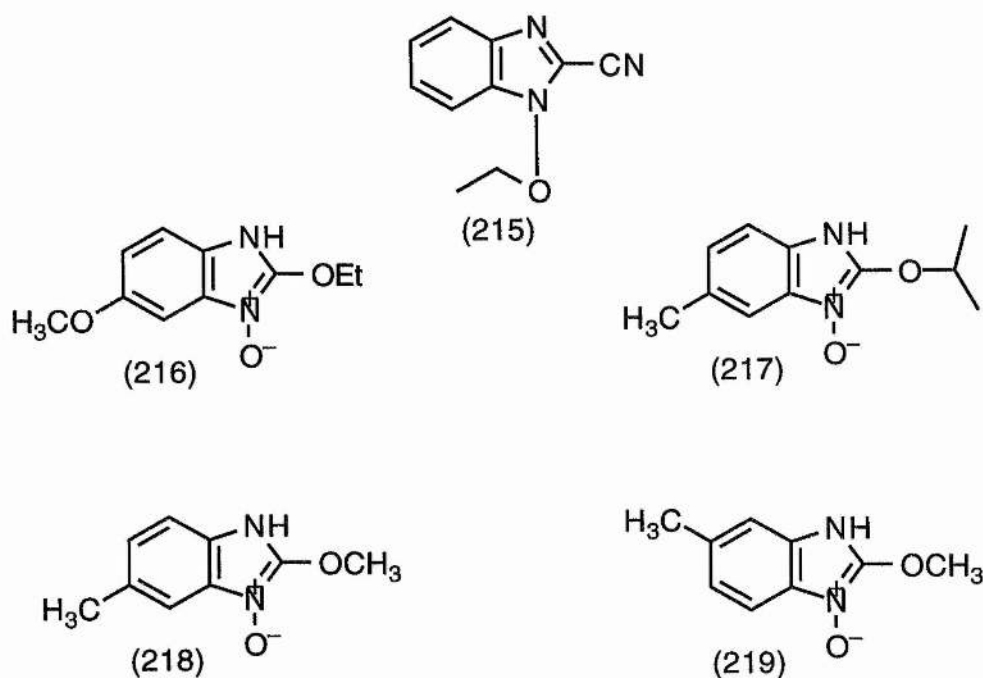
**Table 5.1 Activities of benzimidazole nucleosides against HSV1**

<u>compound</u>	<u>activity <math>\mu</math>M</u>	<u>toxicity</u>
137	—	200
136	—	200
106	25	25
111	—	100
107	—	100

### **5.7 Activity against other viruses**

Several benzimidazole *N*-oxides (some arising from the present work and others from previous investigations), and some benzimidazole- and benzotriazole-based nucleoside analogues arising in the early stages of this project, were submitted for testing against a panel of viruses by staff of the Wellcome Research Laboratories. The compounds were tested against the following viruses/(cell lines): HSV2 (vero), HCMV (MRC5), HIV-1 (HeLa), and papilloma; and they were also tested for toxicity.

The nucleoside analogues [(197), (200), (107), (136) and (111)] and *N*-oxides (see below) tested were as follows:



Just as for the compounds tested 'in-house' against HSV1, these compounds seemed in the majority of cases to possess no activity. Where activity against one of the panel of viruses was found, it was closely accompanied by toxicity in an uninfected cell-line.

Compound (200), which showed neither activity nor toxicity against HSV1, possessed activity against HCMV between 100 and 10  $\mu\text{M}$ , but displayed toxicity at 456  $\mu\text{M}$ . Compound (107) showed activity against HSV2, but as in the case of HSV1 this was due to toxicity at the very low concentration of 116  $\mu\text{M}$ .

The only other compound to display any activity was the *N*-oxide (219), against HCMV and HIV at concentrations between 100 and 10  $\mu\text{M}$ . Again, activity was not well separated from cytotoxicity, 471  $\mu\text{M}$  being the level at which this was displayed.

Several compounds, although not displaying activity at any of the concentrations tested, showed toxicity. Compounds (215), (136) and (111) showed toxicity at 351, 227 and 443  $\mu\text{M}$  respectively.

**Table 5.2 Activity of selected compounds against a panel of viruses**

<u>cpd. no.</u>	<u>HSV2</u>	<u>HCMV</u>	<u>HIV</u>	<u>PAPILLOMA</u>	<u>tox.</u>
197	>100	>100	>100	>100	>500
200	>100	>10<100	>100	<100	456
216	>100	>100	>100	>100	>500
217	>100	>100	>100	>100	>500
218	>100	>100	>100	>100	>500
219	>100	>10<100	>10<100	>100	471
215	>100	>100	>100	<100	351
136	>100	>100	>100	<100	227
107	>10<100	>100	>100	>100	116
111	>100	>100	>100	>100	443

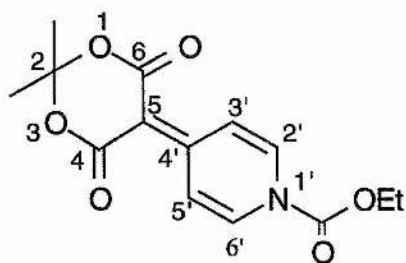
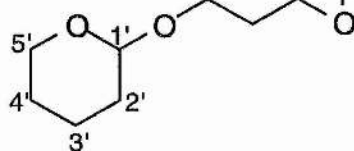
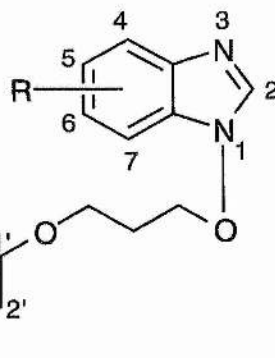
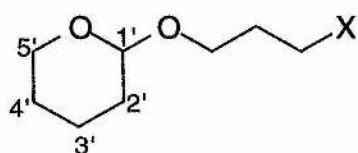
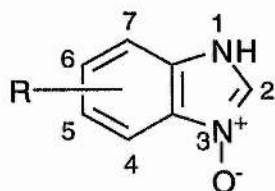
(results given as CC<sub>50</sub> in  $\mu$ M, 96 hours incubation)

## 5.8 Conclusion

The compounds which have been tested against both HSV1 and the Wellcome panel of viruses (HSV2, HCMV, HIV and papilloma) have shown that any activity displayed against these viruses is very closely linked to toxicity against uninfected cells. Very little can be said at this early stage about the relationship between the substituents in these nucleoside analogues and their activity or toxicity. Many more analogues need to be synthesised in order to ascertain if substituents may be added to, or omitted from, the molecule in order to retain the activity while removing the toxicity. The two processes may of course be inseparable: it has been shown, however, that such nucleoside analogues do possess a valid and possibly exploitable biological activity which is open to further investigation.

## Experimental

### Numbering diagrams:





## Chapter 2

### Experimental

IR spectra were recorded on a Perkin Elmer 1710 Infrared Fourier Transform Spectrophotometer, and samples were run as thin films or Nujol mulls;  $^1\text{H}$  nmr spectra were recorded at 200MHz on a Varian Gemini or as specified on a Bruker AM300 at 300MHz, and  $^{13}\text{C}$  nmr spectra at 50MHz or 75MHz, respectively, for solutions in  $d_6$ -DMSO relative to TMS ( $\delta_{\text{H}} = 0$ ,  $\delta_{\text{C}} = 0$ ) and J are given in Hz. Melting points were carried out on a Electrothermal 9100 digital melting point apparatus, and mass spectra were generated under electron impact on an A.E.I. MS-50 spectrometer unless otherwise stated. Column Chromatography was carried using Merck silica gel 60 (230-400 mesh), and tlc using 0.25mm layers of silica gel on glass sheets. Solvents were distilled from calcium hydride when required anhydrous. Petroleum used was the fraction boiling from 40-60 °C. The concentrated mineral acids used were (sp. gr.): hydrochloric, 1.18, sulphuric, 1.83, nitric, 1.5. The concentrated ammonia solution used was sp. gr. 0.88.

In all compounds containing alkoxy or hydroxyalkoxy groups, the vicinal  $^1\text{H}$  coupling constants were approximately 7 Hz.

#### 2,4-Dinitrophenylglycine ethyl ester (86a)

1-Chloro-2,4-dinitrobenzene (85) (50.0g), glycine ethyl ester hydrochloride (34.5g) and sodium bicarbonate (41.6g) were heated under reflux for 2h in ethanol (450ml), the reaction mixture then being allowed to cool and a precipitate forming. The reaction mixture was then poured on to ice and water (500 ml), the precipitate isolated by filtration, washed with ethanol and finally recrystallised from acetic acid: yield 51.4g (78%), m.p.142-143°C (lit.<sup>123</sup> 144°C).  $\nu_{\text{max}}$ . 3340 (N-H),  $1750\text{cm}^{-1}$  (C=O),  $\delta_{\text{H}}$  1.25 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.20 (2H, q,



H-3), 9.05 (1H, t,  $\text{NH-CH}_2$ ),  $J_{5,6}=10.0$ ,  $J_{3,5}=2.5$ ,  $J_{\text{NH-CH}_2}=5.0$ ,  $\delta_{\text{C}}$  14.0 ( $\text{CH}_2\text{CH}_3$ ), 44.6 ( $\text{NH-CH}_2$ ), 61.2 ( $\text{CH}_2\text{CH}_3$ ), 115.8 (C-3), 123.2 (C-6), 129.8 (C-5), 130.1 (C-1), 135.6 (C-2), 147.8 (C-4), 168.9 (C=O).

#### 2,4-Dinitrophenylglycine methyl ester (86b)

1-Chloro-2,4-dinitrobenzene (85) (20.4g), glycine methyl ester hydrochloride (12.6g) and sodium bicarbonate (16.8g) were heated under reflux for 3h in methanol (500 ml) then allowed to cool, a precipitate forming. The above work up procedure was then followed: yield 17.4g (68%), m.p. 118-119°C (lit.<sup>124</sup> 114-115°C),  $\nu_{\text{max}}$  3348 (N-H), 1758 (C=O), 1524 and 1337  $\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  3.85 (3H, s,  $\text{CH}_3$ ), 4.20 (2H, d,  $\text{CH}_2$ ), 6.80 (1H, d, H-6), 8.30 (1H, dd, H-5), 8.95 (1H, bs, NH), 9.15 (1H, d, H-3),  $J_{5,6}=10.0$ ,  $J_{3,5}=2.5$ ,  $J_{\text{CH}_2\text{-NH}}=5.0$ ,  $\delta_{\text{C}}$  44.5 ( $\text{NH-CH}_2$ ), 52.3 ( $\text{CH}_3$ ), 115.8 (C-3), 123.2 (C-6), 129.8 (C-5), 130.2 (C-1), 135.7 (C-2), 147.9 (C-4), 169.4 (C=O).

#### Attempted cyclisation of (86a) to ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a)

Previous to the experimental procedure illustrated below, the cyclisation of 2,4-dinitrophenylglycine ethyl ester (86a) was attempted under a variety of conditions based upon the literature procedure<sup>73</sup>, whereby the base used was piperidine. In each case the yield was very low and changes to the base, reaction time and conditions are tabulated below. The major product isolated from each reaction was shown to be unchanged starting material.

<u>Reaction time/ process change</u>	<u>Base</u>	<u>Yield (%)</u>
1h reflux	piperidine	19
2h reflux	"	31
3h reflux	"	15
base redistilled	"	6
base dried/2h reflux	"	30
different base, (see below)	$\text{K}_2\text{CO}_3$	69

Ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a)

2,4-Dinitrophenylglycine ethyl ester (86a) (49.1g) and potassium carbonate (25.2g) were heated under reflux in ethanol (500ml) for 5h, then allowed to cool. The reaction mixture was partitioned between dichloromethane and water, the water layer being kept and carefully acidified with concentrated hydrochloric acid to yield a yellow precipitate with evolution of CO<sub>2</sub> gas. The precipitate was recrystallised from ethanol: yield 31.5g (69%), m.p.192-194°C (lit.<sup>125</sup> 209-210°C),  $\nu_{\max}$ .1729 (C=O), 1529, 1505 and 1348cm<sup>-1</sup> (NO<sub>2</sub>),  $\delta_{\text{H}}$  1.40 (3H, t, CH<sub>2</sub>CH<sub>3</sub>), 4.50 (2H, q, CH<sub>2</sub>CH<sub>3</sub>), 7.95 (1H, d, H-7), 8.15 (1H, dd, H-6), 8.40 (1H, d, H-4),  $J_{6,7}$ =9.0,  $J_{4,6}$ =3.0,  $\delta_{\text{C}}$  14.2 (CH<sub>2</sub>CH<sub>3</sub>), 62.3 (CH<sub>2</sub>CH<sub>3</sub>), 107.5 (C-4), 118.4 (C-7), 122.1 (C-6), 132.1 (C-3a), 140.8 (C-2), 142.1 (C-5), 144.6 (C-7a), 157.7 (C=O).

Methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b)

2,4-Dinitrophenylglycine methyl ester (86b) (17.4g) and potassium carbonate (9.5g) were heated under reflux in methanol (500 ml) for 2h then allowed to cool. The reaction mixture was then worked up as for (87a), above: yield 4.6g (29%), m.p.198-199°C (lit.<sup>125</sup> 205-206°C),  $\nu_{\max}$ .1733 (C=O), 1525, 1501 and 1354cm<sup>-1</sup> (-NO<sub>2</sub>),  $\delta_{\text{H}}$  4.00 (3H, s, CH<sub>3</sub>), 8.00 (1H, d, H-7), 8.20 (1H, dd, H-6), 8.40 (1H, d, H-4),  $J_{6,7}$ =9.0,  $J_{4,6}$ =3.0,  $\delta_{\text{C}}$  53.3 (CH<sub>3</sub>), 107.4 (C-4), 118.6 (C-7), 122.3 (C-6), 132.2 (C-3a), 140.8 (C-2), 141.9 (C-5), 144.9 (C-7a), 158.0 (C=O),  $m/z$  =237 (M<sup>+</sup>).

As mentioned in the results and discussion, the benzimidazole *N*-oxide may be reached by a variety of routes: below, method A refers to hydrolysis/decarboxylation in concentrated hydrochloric acid and method B to hydrolysis/decarboxylation in sodium hydroxide.

5-Nitrobenzimidazole 3-oxide (88)

Method A (from ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a))

Ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a) (29.0g) was heated under reflux in concentrated hydrochloric acid (725ml) (25ml per 1.0g reactant) for 1.5h then allowed to cool, a precipitate forming. The hydrochloride of (88) was collected by filtration and

recrystallised from ethanol: m.p. 236-242°C (lit.<sup>73</sup> *ca.* 240°C),  $\delta_{\text{H}}$  7.95 (1H, d, H-7), 8.30 (1H, dd, H-6), 8.60 (1H, d, H-4), 9.85 (1H, s, H-2),  $J_{6,7}=10.0$ ,  $J_{4,6}=3.0$ ,  $\delta_{\text{C}}$  (300MHz) 107.4 (C-4), 118.4 (C-7), 119.2 (C-6), 129.7 (C-3a), 137.8 (C-5), 142.8 (C-2), 144.1 (C-7a). The hydrochloride salt of (88) was then stirred in aqueous ammonia (*d* 0.88; 40ml per 1g of product) (600ml) for approximately 20 minutes and the solution then concentrated to precipitate a yellow solid: yield 11.8g (64%), m.p. 276-278°C (lit.<sup>73</sup> 274-276°C),  $\nu_{\text{max}}$ . 1560, 1400, 1350, 1338 and 1318 $\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  7.60 (1H, d, H-7), 7.85-7.93 (2H, m, H-6, H-4), 8.40 (1H, s, H-2),  $J_{6,7}=10.0$ ,  $\delta_{\text{C}}$  (300MHz) 106.2 (C-4), 116.0 (C-7), 119.7 (C-6), 131.0 (C-3a), 142.1 (C-5), 143.4 (C-7a), 144.4 (C-2).

Method A (from methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b))

Methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b) (3.5g) was reacted in concentrated hydrochloric acid following the above procedure including the reaction in ammonia solution: yield 2.2g (84%), m.p. 267-268°C (lit.<sup>73</sup> 274-276°C)  $\nu_{\text{max}}$ . 1560, 1400, 1350, 1338 and 1318 $\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  7.63 (1H, d, H-7), 7.86 (2H, m, H-6, H-4), 8.43 (1H, s, H-2),  $J_{6,7}=10.0$ ,  $J_{4,6}=2.5$ ,  $\delta_{\text{C}}$  (300MHz) 106.4 (C-4), 115.8 (C-7), 119.8 (C-6), 131.2 (C-3a), 141.8 (C-5), 143.4 (C-7a), 144.2 (C-2).

Method B (from ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a))

Ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a) (0.25g) was stirred at 50 °C in sodium hydroxide (2M, 10ml) and water (10ml) for 4h. Acidification of the cooled reaction mixture yielded a yellow precipitate which was collected by filtration and washed with water. This was tentatively identified as the hydrochloride salt of the expected product: yield 0.2g (93%), m.p. 240-242°C (decomp.) (lit.<sup>73</sup> *ca.* 240°C),  $\nu_{\text{max}}$ . 1560, 1400, 1350, 1338 and 1318 $\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  7.85 (1H, d, H-7), 8.10 (1H, dd, H-6), 8.36 (1H, d, H-4), 8.78 (1H, s, H-2),  $J_{6,7}=10.0$ ,  $J_{4,6}=2.5$ ,  $m/z$  =179 ( $\text{M}^{+}$ )

Method B (from methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b))

Methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b) (0.5g) was dissolved in sodium hydroxide (2M, 15ml) and stirred at room temperature for 30 minutes. The precipitate formed was collected by filtration, dissolved in water and acidified with concentrated hydrochloric acid. The solid was collected and recrystallised from ethanol: yield 0.2g, m.p. 216-217°C (decomp.) (lit.<sup>73</sup> 240°C),  $m/z$  = 179 ( $M^+$ ), The  $^1\text{H}$  nmr spectrum indicated that the product was probably a mixture of the *N*-oxide and its hydrochloride; this reaction was not investigated further.

Attempted formation of 1-ethoxy-6-nitrobenzimidazole (90)

To a stirred solution of 5-nitrobenzimidazole 3-oxide (88) (0.5g) dissolved in ethanol (50ml), a solution containing 1 equivalent of sodium ethoxide in ethanol (25ml) was added, and stirring continued for a further 1h over which time a precipitate developed. Addition of extra ethanol (50ml) and ethyl iodide (0.44g) with continued stirring saw the precipitate persist. The precipitate was isolated by filtration and shown to be the sodium salt of the anion of (88).

Success was achieved in this reaction via the use of DMF as the solvent, but yields were still fairly low. Formation of the anion in the reaction medium was next carried out by the addition of the sodium ethoxide solution, removal of the ethanol solvent after stirring for 1h, dissolution of the residue in DMF and addition of the ethyl iodide. Improvement in the yield was finally realised by the addition of solid sodium ethoxide to a solution of the starting material (88) in DMF, whereupon the formation of the anion in solution gave rise to a deep red colour. Traces of starting material were still present in the final product; these were removed by washing the organic layers in the work-up with aqueous sodium hydroxide followed by washing with water.

The best methodology for the reaction is cited overleaf:

1-Ethoxy-6-nitrobenzimidazole (90)

To a stirred solution of 5-nitrobenzimidazole 3-oxide (88) (2.1g) in DMF (50ml) was added solid sodium ethoxide (0.8g), giving a red solution. Ethyl iodide (1.83g) was then added, and stirring was continued for 12h. The reaction mixture was then poured into water (100ml) and extracted three times with dichloromethane (3x50ml); the extracts were combined and washed with sodium hydroxide (0.1M, 50ml) followed by three washes with water (3x150ml) and drying over magnesium sulphate. Concentration of the organic layers yielded a yellow solid which was recrystallised from ethanol: yield 0.6g (23%), m.p.99-100°C. (Found: C, 52.5; H, 4.3; N, 20.7.  $C_9H_9N_3O_3$  requires C, 52.2; H, 4.35; N, 20.3%),  $\nu_{\max}$ . 1510 and  $1343\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) 1.40 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.50 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 7.90 (1H, d, H-4), 8.20 (1H, dd, H-5), 8.30 (1H, d, H-7), 8.45 (1H, s, H-2),  $J_{4,5}=10.0$ ,  $J_{5,7}=2.5$ ,  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) (300MHz) 13.8 ( $\text{CH}_2\text{CH}_3$ ), 76.6 ( $\text{CH}_2\text{CH}_3$ ), 105.9 (C-7), 118.3 (C-5), 121.3 (C-4), 129.4 (C-3a), 142.7 (C-2), 143.4 (C-7a), 144.4 (C-6),  $m/z = 207$  ( $\text{M}^+$ ).

2-Methoxy-5-nitrobenzimidazole (103) from the attempted formation of 1-ethoxy-6-nitrobenzimidazole (90)

Sodium methoxide (0.20g) was added to a solution of 5-nitrobenzimidazole 3-oxide (88) (0.32g) in DMF (30ml), the solution becoming dark red in colour. Ethyl iodide (0.30g) was then added, stirring being maintained overnight. The reaction mixture was then poured into water (300ml) and extracted with dichloromethane (3x50ml); the organic extracts were combined, washed with water (150ml), dried over magnesium sulphate, filtered and concentrated to yield a yellow solid which was recrystallised from methanol: yield 0.20g (47%), m.p.186-187°C,  $\delta_{\text{H}}$  4.15 (3H, s,  $\text{CH}_3$ ), 7.50 (1H, d, H-7), 8.0 (1H, dd, H-6), 8.15 (1H, s, H-4),  $J_{4,5}=10.0$ ,  $J_{5,7}=2.5$ .

No further spectroscopic data were gathered, the assignment being tentatively made on the evidence of the  $^1\text{H}$  nmr and the knowledge of the possible addition-elimination reaction. Further use of the product was not envisaged.

Ethyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89a)

Ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a) (0.5g) was dissolved in DMF (10ml) and added with stirring to a solution of DMF (25ml) containing sodium ethoxide (0.16g). Ethyl iodide (0.35g) was then added and stirring maintained for 1.5h. The solvent was removed by evaporation, the yellow solid was washed with water and recrystallised from ethanol: yield 0.3g (57%), m.p. 121-122°C. (Found: C, 51.8; H, 4.3; N, 14.9.  $C_{12}H_{13}N_3O_5$  requires C, 51.6; H, 4.7; N, 15.05%),  $\nu_{\max}$  1742 (C=O), 1560, 1519, 1501, 1349 and  $1319\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) (300MHz), 1.53 and 1.58 (6H, 2t,  $2\text{CH}_2\text{CH}_3$ ), 4.58 and 4.63 (2H, 2q,  $2\text{CH}_2\text{CH}_3$ ), 8.02 (1H, d, H-4), 8.20 (1H, dd, H-5), 8.60 (1H, d, H-7),  $J_{4,5}=11.0$ ,  $J_{5,7}=3.0$ ,  $\delta_{\text{C}}$  13.7 ( $\text{CH}_2\text{CH}_3$ ), 14.3 ( $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 69.1 ( $\text{CH}_2\text{CH}_3$ ), 76.9 ( $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 107.1 (C-7), 119.4 (C-4), 122.9 (C-5), 131.3 (C-7a), 140.8 (C-2), 141.5 (C-6), 145.8 (C-3a), 157.2 (C=O),  $m/z = 279$  ( $\text{M}^{+}$ ).

Attempted synthesis of methyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89b)

Methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b) (1.5g) was dissolved in DMF (50ml) and sodium ethoxide (0.4g) in DMF (30ml) added, the solution turning dark red. Ethyl iodide (0.9g) was then added, and the reaction left to stir overnight, after which time the dark red colour had returned to the initial pale yellow of the starting solution. After isolation and characterisation the product was shown to be a mixture of methyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate and ethyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate, exchange having occurred between the ester and the anion of the base.

Methyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89b)

Methyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87b) (1.5g) was dissolved in DMF (50ml) before addition, with stirring of a solution of sodium methoxide (0.3g) in DMF (30ml). Ethyl iodide (0.94g) was then added, the reaction being left to stir overnight (ca. 12h), after which time the dark red colour of the anion in solution had returned to a pale yellow. The reaction mixture was poured into water (100ml) then extracted twice with dichloromethane (2x75ml). The organic extracts were combined and washed with water (150ml), dried over



magnesium sulphate, filtered and concentrated *in vacuo* to a dark solid. This was recrystallised from methanol with charcoal to yield a white solid: yield 0.04g (3%), m.p. 125-129°C. (Found: C, 50.0; H, 4.15; N, 16.1.  $C_{11}H_{11}N_3O_5$  requires C, 49.8; H, 4.15; N, 15.85%).  $\nu_{\max}$ , 1734 (C=O), 1515, 1502, 1349 and 1311  $cm^{-1}$  ( $NO_2$ ),  $\delta_H$  ( $CDCl_3$ ) 1.60 (3H, t,  $CH_2CH_3$ ), 4.15 (3H, s,  $CH_3$ ), 4.65 (2H, q,  $CH_2CH_3$ ), 7.95 (1H, d, H-4), 8.25 (1H, dd, H-5), 8.50 (1H, d, H-7),  $J_{4,5}=10.0$ ,  $J_{5,7}=2.5$ ,  $\delta_C$  ( $CDCl_3$ ) 13.7 ( $CH_2CH_3$ ), 31.0 ( $CH_2CH_3$ ), 53.6 ( $CO_2CH_3$ ), 107.1 (C-7), 119.4 (C-4), 122.9 (C-5), 131.3 (C-7a), 140.8 (C-2), 141.2 (C-6), 145.9 (C-3a), 157.6 (C=O).

As mentioned previously, method A refers to hydrolysis/decarboxylation in concentrated hydrochloric acid and method B to hydrolysis/decarboxylation in sodium hydroxide, as cited earlier.

#### 1-Ethoxy-6-nitrobenzimidazole (90)

Method A (from ethyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89a))

Ethyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89a) (0.3g) was heated under reflux in concentrated hydrochloric acid (10ml) for 1.5h. After cooling, concentrated ammonia was added with stirring before concentration *in vacuo* to yield a pale yellow solid: yield 0.3g, m.p. ca. 300°C (decomp.),  $\nu_{\max}$ , 1510  $cm^{-1}$  and 1343  $cm^{-1}$  ( $NO_2$ ),  $\delta_H$  ( $CDCl_3$ ) 1.38 (3H, t,  $CH_2CH_3$ ), 4.50 (2H, q,  $CH_2CH_3$ ), 7.91 (1H, d, H-4), 8.16 (1H, dd, H-5), 8.54 (1H, d, H-7), 9.01 (1H, s, H-2),  $J_{4,5}=10.0$ ,  $J_{5,7}=2.5$ ,  $m/z = 208$  ( $M+1^+$ ).

The very high melting point and downfield position of H-2 in the  $^1H$  nmr of this sample may indicate that the product isolated here is in fact the ammonium salt of the anion; but the success of method B (below) made further investigation of method A unnecessary.

Method B (from ethyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89a))

Ethyl 1-ethoxy-6-nitrobenzimidazole-2-carboxylate (89a) (1.0g) was dissolved in DMF (30ml), to give a pale yellow solution. Sodium hydroxide (2M, 40ml) was then added, the solution turning a deep red colour followed by immediate precipitation. The reaction was then

heated to around 50°C and maintained at this temperature for 2h. On cooling, the precipitate reformed but disappeared when the reaction mixture was poured into water (300ml). Acidification with concentrated hydrochloric acid precipitated a product. The aqueous layer was further extracted with dichloromethane (50ml), the extracts being combined, washed with water (50ml), dried over magnesium sulphate, then filtered and concentrated to leave a yellow solid. The product was then recrystallised from ethanol: yield 0.6g (77%), m.p. 100-102°C;  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) 1.50 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.45 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 7.85 (1H, d, H-4), 8.20 (1H, dd, H-5), 8.30 (1H, d, H-7), 8.40 (1H, s, H-2),  $J_{4,5}=10.0$ ,  $J_{5,7}=2.5$ ,  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 13.9 ( $\text{CH}_2\text{CH}_3$ ), 76.6 ( $\text{CH}_2\text{CH}_3$ ), 106.0 (C-7), 118.3 (C-4), 121.3 (C-5), 142.9 (C-2), 143.5 (C-6), 144.3 (C-3a).

#### 2-(3-Bromopropoxy)tetrahydropyran (105)

##### Pyridinium *p*-toluenesulphonate (PPTS)

*p*-Toluenesulphonic acid (5.70g) was dissolved in an excess of pyridine (20ml) and stirred for approximately 20 minutes, the excess pyridine then being removed *in vacuo*. The white solid collected was recrystallised from acetone: yield 4.7g (56%), m.p. 119-120°C (lit.<sup>84</sup> 120°C).

To a solution of 2,3-dihydropyran (9.2g) and 3-bromopropan-1-ol (104) (10g) in anhydrous dichloromethane (250ml), PPTS (1.8g) was added with stirring. Stirring was continued for 24h, the colour changing through light green to a very dark green. The solution was then diluted to approximately 750ml with ether, extracted once with half-saturated brine and finally concentrated to a brown oil *in vacuo*. The crude product was distilled to yield the pure product as a yellow oil: the oil darkened upon sitting in the atmosphere, but  $^1\text{H}$  nmr showed no evidence of decomposition despite this colour change: yield 13.6g (56%), b.p. 75°C / 2 mmHg (lit.<sup>126</sup> 69°C / 1.2 mmHg),  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) 1.35-1.84 (6H, m, H-2', H-3', H-4'), 2.05 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.43 (2H, t,  $\text{CH}_2\text{Br}$ ), 3.49 (2H, t,  $\text{THPO-CH}_2$ ), 3.79 (2H, m, H-5'), 4.55 (1H, s, H-1'),  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 19.3 (C-3'), 25.3 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 30.4 (C-4'), 32.5 (C-2'), 59.7 ( $\text{CH}_2\text{Br}$ ), 62.0 ( $\text{THPO-CH}_2$ ), 64.7 (C-5'), 98.6 (C-1'),  $m/z = 223$  ( $\text{M}^+$ ).



Attempted production of 1-[3-(tetrahydropyran-2-yloxy)propoxy]-6-nitrobenzimidazole (106)

5-Nitrobenzimidazole 3-oxide (88) (0.5g) was dissolved in sodium ethoxide solution (2.5ml) [sodium (1.0g) dissolved in ethanol (30ml)] and ethanol (10ml), stirred for several minutes and concentrated *in vacuo*. The solid was dissolved in the minimum of DMF (10ml) and 2-(3-bromopropoxy)tetrahydropyran (105) (0.69g) added, stirring at room temperature being maintained for 24h. Attempts were made to purify the materials isolated from the reaction by distillation but on analysis these were found to be mainly the starting THP ether (105). A second attempt at the reaction looked a little more promising, but chromatography of the product mixture on a column of silica gel again yielded none of the desired product. Success was only achieved when the base used was changed to potassium carbonate.

1-[3-(Tetrahydropyran-2-yloxy)propoxy]-6-nitrobenzimidazole (106)

5-Nitrobenzimidazole 3-oxide (88) (1.3g) was dissolved in DMF (35ml) before the addition of potassium carbonate (1.3g). 2-(3-Bromopropoxy)tetrahydropyran (105) (2.0g) was then added, with stirring, at room temperature, and the stirring continued for 24h. The reaction mixture was poured into water (100ml), extracted with dichloromethane (3x50ml), the organic layers combined, washed with water and dried over magnesium sulphate. Concentration of the organic layers yielded a pale yellow solid. The product was purified on a column of silica and recrystallised from toluene: yield 0.55g (20%), m.p.68-70°C. (Found: C, 55.8; H, 6.0; N, 12.8.  $C_{15}H_{19}N_3O_5$  requires C, 56.1; H, 6.0; N, 13.1%),  $\delta_H$  1.47-1.75 (6H, m, H-2', H-3', H-4'), 2.06 (2H, quin.,  $CH_2-CH_2-CH_2$ ), 3.63 (2H, m,  $CH_2-OTHP$ ), 3.85 (2H, m, H-5'), 4.54 (2H, t,  $CH_2-O-N$ ), 4.64 (1H, d, H-1'), 7.89 (1H, d, H-4), 8.14 (1H, dd, H-5), 8.55 (1H, d, H-7), 8.99 (1H, s, H-2),  $J_{4,5}=8.8$ ,  $J_{5,7}=2.2$ ,  $\delta_C$  18.4 (C-2'), 25.1 (C-4'), 28.1 (C-3'), 30.4 ( $CH_2-CH_2-CH_2$ ), 61.7 (THPO- $CH_2$ ), 62.9 (C-5'), 77.9 (N-O- $CH_2$ ), 98.4 (C-1'), 106.1 (C-7), 117.8 (C-4), 120.9 (C-5), 129.5 (C-3a), 143.1 (C-6), 143.6 (C-2), 144.7 (C-7a).

(C-1'), 106.1 (C-7), 117.8 (C-4), 120.9 (C-5), 129.5 (C-3a), 143.1 (C-6), 143.6 (C-2), 144.7 (C-7a).

Attempted production of 1-(3-Hydroxypropoxy)-6-nitrobenzimidazole (107)

1-[3-(Tetrahydropyran-2-yloxy)propoxy]-6-nitrobenzimidazole (106) (0.25g) was dissolved in ethanol and PPTS (0.03g) added. The reaction mixture was then stirred at 55°C and monitored by tlc. After 3.5h the starting material had all diminished and the solvent was evaporated off to yield a gum. Purification of the highly polar product was attempted on a column of silica, eluted with 10% methanol / 90% ethyl acetate, but the product remained at the baseline. Attempted purification by distillation under high vacuum only furnished carbonaceous material, the product being too thermally unstable.

3-Benzoyloxy-1-bromopropane (108)

from 3-bromopropan-1-ol (104)

3-Bromopropan-1-ol (104) (5.0g), benzyl chloride (15.9g) and powdered potassium hydroxide (6.0g) were heated under reflux in a solution of toluene (65ml) and 1,4-dioxan (18ml) for 5h. The reaction mixture was allowed to cool before the addition of water (200ml), followed by acetic acid, to pH 7. The organic layer was separated, washed with water and dried over magnesium sulphate, followed by concentration *in vacuo* at high temperature to distil off any excess benzyl chloride. The residue was dissolved in a mixture of ethanol (50ml) and hydrochloric acid (0.02M, 50ml) and heated under reflux for a further 2h then allowed to cool. The solution was concentrated to half volume, neutralised with sodium bicarbonate and extracted with dichloromethane (3x50ml). The extracts were combined, washed with water, dried over magnesium sulphate and finally concentrated to a yellow oil. The pure product was obtained by distillation on the Kugelrohr at low pressure: yield 1.0g (12%), b.p. 135°C / 2 mmHg, (lit.<sup>86</sup> 130°C / 5 mmHg),  $\delta_{\text{H}}$  1.88 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.59 and 3.63 (4H, 2t,  $\text{CH}_2\text{-O}$  and  $\text{CH}_2\text{-Br}$ ), 4.54 (2H, s,  $\text{PhCH}_2$ ), 7.35 (5H, s, Ph).

from 3-benzyloxypropan-1-ol (109)

A mixture of 3-benzyloxypropan-1-ol (109) (0.5g) and triphenylphosphine (0.79g) was cooled to  $\sim 5^{\circ}\text{C}$  in an ice water bath with stirring. *N*-Bromosuccinimide (0.54g) was then added portionwise, while keeping the temperature constant. Stirring was continued for 24h. The reaction mixture was filtered, the filtrate then being washed several times with toluene, the toluene fractions were combined and washed with 5% sodium thiosulphate (25ml), sodium hydroxide (0.1M, 2 x 25ml) and brine (25ml), dried over  $\text{MgSO}_4$  and concentrated to an oily residue. The product was distilled at low pressure on the Kugelrohr: yield 0.40g (58%), b.p.  $100^{\circ}\text{C} / 2 \text{ mmHg}$ , (lit.<sup>86</sup>  $130^{\circ}\text{C} / 5 \text{ mmHg}$ ),  $\delta_{\text{H}}$  2.01 (2H, q,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.52 and 3.59 (4H, 2t,  $\text{CH}_2\text{-Br}$ ,  $\text{O-CH}_2$ ), 4.48 (2H, s,  $\text{CH}_2\text{-Ph}$ ), 7.32 (5H, s, Ph).

1-(3-Benzyloxypropoxy)-6-nitrobenzimidazole (110)

5-Nitrobenzimidazole 3-oxide (88) (0.5g) was dissolved in sodium ethoxide solution (2.5ml of a solution made from 1.0g of sodium dissolved in 40ml of ethanol) and stirred for 30 minutes before concentration and dissolution in DMF. 3-Benzyloxy-1-bromopropane (108) (0.4g) was then added with stirring and the stirring continued for 24h. The reaction mixture was poured into water (75ml) and extracted with dichloromethane (3x50ml). The organic layers were combined, washed with water (150ml), dried over magnesium sulphate and concentrated to a yellow oil. Attempts to purify the product, which was detected by  $^1\text{H}$  nmr, on a column of silica proved fruitless, as did the attempted distillation as (110) was too thermally unstable.

1-(3-Hydroxypropoxy)-6-nitrobenzimidazole (107)

5-Nitrobenzimidazole 3-oxide (88) (1.1g) was dissolved in sodium ethoxide solution (5ml of a solution made from 1.0g of sodium dissolved in 40ml of ethanol) and stirred for a 0.5h. The ethanol was evaporated off to yield a orange solid that was then dissolved in DMF (20ml). 3-Bromopropan-1-ol (0.85g) was then added and the reaction mixture stirred at room temperature for 24h, the dark red colour changing to yellow over this period. The reaction mixture was poured into water (150ml) then extracted with dichloromethane (3x75ml). The

dichloromethane layers were combined, washed with sodium hydroxide solution (2M, 50ml), water, dried over magnesium sulphate and concentrated to a yellow solid which was recrystallised from propan-1-ol: yield 0.5g (40%), m.p.120-121°C, (Found: C, 50.7; H, 4.5; N, 17.5.  $C_{10}H_{11}N_3O_4$  requires C, 50.6; H, 4.7; N, 17.7%),  $\delta_H$  (300MHz) 2.10 (2H, quin.  $CH_2-CH_2-CH_2$ ), 3.81 (2H, q,  $CH_2-OH$ ), 4.54 (2H, t,  $CH_2-O-N$ ), 4.79 (1H, t,  $-OH$ ), 7.99 (1H, d, H-4), 8.18 (1H, dd, H-5), 8.55 (1H, d, H-7), 8.98 (1H, s, H-2);  $J_{4,5}=9.6$ ,  $J_{5,7}=1.9$ ,  $J_{HO,CH_2}=4.5$ Hz,  $\delta_C$  30.9 ( $CH_2-CH_2-CH_2$ ), 56.9 ( $HO-CH_2$ ), 77.9 ( $CH_2-O-N$ ), 106.0 (C-7), 117.6 (C-4), 120.8 (C-5), 129.1 (C-7a), 143.0 (C-6), 143.6 (C-3a), 144.4 (C-2),  $m/z = 237$  ( $M^+$ ).

Ethyl 1-(3-hydroxypropoxy)-6-nitrobenzimidazole-2-carboxylate (111)

Ethyl 5-nitrobenzimidazole-2-carboxylate 3-oxide (87a) (2.5g) was dissolved in the minimum of dried DMF (50ml) with the exclusion of moisture (silica gel) and the mixture stirred for 18h. Potassium carbonate (1.5g) and 3-bromopropan-1-ol (1.5g) were added. After addition of the base, the reaction mixture turned a deep red colour which disappeared in time. The reaction mixture was poured into water (200 ml), extracted with dichloromethane (3x75ml), and the organic layers combined and washed with water (2x200ml). The solution was then dried over magnesium sulphate and evaporated to a yellow solid which was recrystallised from toluene: yield 1.6g (52%), m.p.129-131°C, (Found: C, 50.8; H, 4.75; N, 13.8.  $C_{13}H_{15}N_3O_6$  requires C, 50.5; H, 4.9; N, 13.6%),  $\delta_H$  1.40 (3H, t,  $CH_2CH_3$ ), 2.03 (2H, quin.,  $CH_2-CH_2-CH_2$ ), 3.69 (2H, t,  $CH_2-OH$ ), 4.46 (2H, q,  $CH_2CH_3$ ), 4.55 (2H, t,  $CH_2-O-N$ ), 4.48 (1H, bs,  $CH_2-OH$ ), 7.92 (1H, d, H-4), 8.13 (1H, dd, H-5), 8.53 (1H, d, H-7),  $J_{4,5}=8.8$ ,  $J_{5,7}=2.5$ ,  $J_{CH_2,CH_3}=7.0$ ,  $J_{CH_2,OH}=5.0$ ,  $J_{CH_2,CH_2}=6.2$ ,  $\delta_C$  13.8 ( $CH_2CH_3$ ), 30.9 ( $CH_2-CH_2-CH_2$ ), 57.1 ( $HO-CH_2$ ), 62.4 ( $CH_2CH_3$ ), 78.0 ( $CH_2-O-N$ ), 107.2 (C-7), 118.8 (C-4), 122.4 (C-5), 130.7 (C-3a), 140.5 (C-2), 141.5 (C-6), 145.3 (C-7a), 156.8 (C=O).

### 6-Amino-1-ethoxy-benzimidazole (113)

6-Amino-1-ethoxy-benzimidazole (90) (0.05g) was dissolved in ethanol (100ml), palladium/carbon catalyst (5%, 75mg) was added and the flask evacuated of air and stirring continued under an atmosphere of hydrogen. After hydrogen uptake had ceased (*ca.* 175ml hydrogen absorbed), the catalyst was removed by filtration and the solvent evaporated off to yield a dark brown, viscous oil. The product was purified on a column of silica gel, the pure product being a yellow/brown solid: yield 0.05g (9%), m.p. 98-100°C,  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) 1.35 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.30 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 5.20 (2H, bs,  $\text{NH}_2$ ), 6.60 (2H, m, H-5 and H-7), 7.30 (1H, d, H-4), 8.15 (1H, s, H-2),  $J_{4,5}=10$ ,  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 14.0 ( $\text{CH}_2\text{CH}_3$ ), 75.1 ( $\text{CH}_2\text{CH}_3$ ), 93.4 (C-7), 112.9 (C-5), 121.5 (C-4), 131.2 (C-3a), 133.2 (C-7a), 136.9 (C-2), 143.8 (C-6),  $m/z = 177$  ( $\text{M}^+$ ).

Upon storage the product (113) darkened in colour and further purification yielded no product. The hydrochloride salts of the *N*-oxides were easier to handle and isolate hence in the following reaction the reaction mixture was treated with hydrogen chloride gas prior to the work-up procedure.

### 6-Amino-1-ethoxy-benzimidazole hydrochloride (114)

1-Ethoxy-6-nitrobenzimidazole (90) (0.7g) was dissolved in ethanol (50ml), the palladium on carbon catalyst (100mg) was added and stirring continued under an atmosphere of hydrogen for 12h. The catalyst was filtered off, and the solution was then saturated with hydrogen chloride gas resulting in decolourisation. Upon standing, a precipitate formed and was collected; on further standing a second crop developed which was also collected. Spectroscopic analysis showed these to be different products: crop 1 was found to be 5-aminobenzimidazole hydrochloride (115), yield 0.30g (53%), m.p. 240°C (decomp.),  $\delta_{\text{H}}$  7.55 (1H, dd, H-6), 7.95 (2H, d, H-7, H-4), 9.70 (1H, s, H-2). Crop 2: 1-ethoxy-6-aminobenzimidazole hydrochloride (114); 0.1g (14%), m.p. 186-188°C,  $\delta_{\text{H}}$  1.12 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.28 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 7.20 (1H, dd, H-5), 7.55 (1H, d, H-7), 7.65 (1H, d, H-4), 9.45 (1H, s, H-2), 8.13-8.88 (2H, bs,  $\text{NH}_2$ ).



Spectroscopic analysis showed these to be different products: crop 1 was found to be 5-aminobenzimidazole hydrochloride (115), yield 0.30g (53%), m.p.240°C (decomp.),  $\delta_{\text{H}}$  7.55 (1H, dd, H-6), 7.95 (2H, d, H-7, H-4), 9.70 (1H, s, H-2). Crop 2: 1-ethoxy-6-aminobenzimidazole hydrochloride (114); 0.1g (14%), m.p.186-188°C,  $\delta_{\text{H}}$  1.12 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.28 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 7.20 (1H, dd, H-5), 7.55 (1H, d, H-7), 7.65 (1H, d, H-4), 9.45 (1H, s, H-2), 8.13-8.88 (2H, bs,  $\text{NH}_2$ ).

#### Attempted hydrogenation to 5-aminobenzimidazole 3-oxide hydrochloride

5-Nitrobenzimidazole 3-oxide hydrochloride (0.6g) (88) was dissolved in two equivalents of sodium hydroxide solution (2M, 3ml) and water (5ml). The palladium catalyst (5% on carbon) was then added and the reaction mixture left stirring over 18h under an atmosphere of hydrogen. Removal of the catalyst was followed by the acidification of the reaction mixture with concentrated hydrochloric acid and evaporation of the water *in vacuo*. Analysis showed only unreacted starting material to have been returned.

#### Attempted hydrogenations of 1-(3-hydroxypropoxy)-6-nitrobenzimidazole (107)

1-(3-Hydroxypropoxy)-6-nitrobenzimidazole (107) (0.1g) was dissolved in a mixture of propan-1-ol (25ml) and ethanol (25ml), the palladium on carbon catalyst (100mg) was added and stirring continued under an atmosphere of hydrogen for 18h. The solution was saturated with hydrogen chloride gas after removal of the catalyst. The solvent was removed *in vacuo*.  $^1\text{H}$  nmr analysis of the crude isolate showed it to be 5-aminobenzimidazole hydrochloride (115)<sup>127</sup>.

1-(3-Hydroxypropoxy)-6-nitrobenzimidazole (107) (0.1g) was dissolved in formic acid (10ml, 80%), the palladium on carbon catalyst (100mg) added and stirring was continued under an atmosphere of hydrogen for 45 minutes. The catalyst was removed by filtration and the solution concentrated *in vacuo*. The residue was suspended in a solution of water (10ml) and ammonia solution (1.0ml) and heated under reflux for 15 minutes. After cooling the solvent was removed *in vacuo*.  $^1\text{H}$  nmr analysis of the crude isolate showed it to be 5-aminobenzimidazole hydrochloride (115)<sup>127</sup>.

filtered hot and the residue washed with hot methanol. Evaporation of the solvent and analysis of the crude product by tlc (which showed many spots) and  $^1\text{H}$  nmr showed no discernable product or evidence of unchanged starting material.

1-(3-Hydroxypropoxy)-6-nitrobenzimidazole (0.1g) (107) was dissolved in a solution of titanous chloride in hydrochloric acid (12.5% w/v, 3ml) and the flask evacuated at the water pump to yield a clear solution. The reaction mixture was then shaken for 24h followed by basification with ammonia solution and extraction with dichloromethane (3x75ml). The dichloromethane layers were combined, washed with water (150ml), dried with magnesium sulphate and concentrated to a yellow solid which was found to be unreacted starting material (107).

#### 4-Fluoro-3-nitroacetanilide (117)

4-Fluoro-3-nitroaniline (116) (1.0g) was stirred in acetic anhydride (1.96g) for 1h at room temperature. The reaction mixture was then poured onto ice and water (100ml). The brown solid formed was collected by filtration and recrystallised from aqueous ethanol (with charcoal), to yield a pale yellow crystalline solid: yield 1.1g (87%), m.p. 138-140°C (lit.<sup>87</sup> 139°C),  $\delta_{\text{H}}$  2.10 (3H, s,  $\text{COCH}_3$ ), 7.54 (1H, m, H-6), 7.84 (1H, dd, H-5), 8.52 (1H, t, H-2),  $J_{5,\text{F}}=12.0$ ,  $J_{5,6}=9.0$ ,  $J_{2,6}=2.0$ ,  $J_{2,\text{F}}=2.0$ ,  $\delta_{\text{C}}$  24.2 ( $\text{CH}_3$ ), 115.2 (C-2), 118.2 (C-5), 126.1 (C-6), 136.2 (C-1,3), 150.0 (C-4), 168.8 (C=O),  $J_{4,\text{F}}=257.9$ ,  $J_{6,\text{F}}=8.0$ ,  $J_{5,\text{F}}=22.0$ ,  $J_{2,\text{F}}=2.1$ ,  $m/z = 198$  ( $\text{M}^+$ ).

#### N-(4-Acetamido-2-nitrophenyl)glycine ethyl ester (118)

4-Fluoro-3-nitroacetanilide (117) (12.5g), glycine ethyl ester hydrochloride (9.7g) and sodium bicarbonate (10.6g) were heated in DMSO (40ml) with stirring at approximately 50°C for 6.5h; as time progressed the reaction mixture became dark red in colour. The reaction mixture was poured onto ice and water (500ml) to yield a red precipitate which was collected by filtration. The solid was recrystallised from ethanol to yield an orange crystalline solid: yield 7.7g (43%), m.p. 162-164°C (lit.<sup>87</sup> 164-165°C),  $\delta_{\text{H}}$  1.24 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 2.04 (3H, s,  $\text{CH}_3$ ), 4.19 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 4.26 (2H, d,  $\text{NH-CH}_2$ ), 6.93 (1H, d, H-6), 7.66 (1H, dd,

H-5), 8.30 (1H, t,  $\text{NH-CH}_2$ ), 8.51 (1H, d, H-3), 10.02 (1H, s, NH),  $J_{5,6}=9.3$ ,  $J_{3,5}=2.4$ ,  $J_{\text{CH}_2,\text{CH}_3}=7.2$ ,  $J_{\text{NH},\text{CH}_2}=5.4$ ,  $\delta_{\text{C}}$  14.2 ( $\text{CH}_2\text{CH}_3$ ), 23.9 ( $\text{CH}_3$ ), 44.4 ( $\text{NH-CH}_2$ ), 61.1 ( $\text{CH}_2\text{CH}_3$ ), 115.1 (C-5) and (C-6), 128.7 (C-4), 129.4 (C-3), 130.6 (C-2), 141.3 (C-1), 168.4 ( $\text{CH}_3\text{CO}$ ), 170.0 ( $\text{C=O}$ ),  $m/z = 281$  ( $\text{M}^+$ ).

Ethyl 5-acetamidobenzimidazole-2-carboxylate 3-oxide (119)

4-Acetamido-2-nitrophenylglycine ethyl ester (118) (6.0g) and potassium carbonate (3.2g) were heated together in ethanol (75ml) under reflux for 8.5h. The solvent was then evaporated off to yield a dark red solid which was dissolved in the minimum water and carefully acidified to pH 1 with concentrated hydrochloric acid. A precipitate was collected and recrystallised from ethanol and water to yield an orange crystalline solid: yield 3.7g (66%), m.p. 133-138°C (lit.<sup>87</sup> 133-134°C),  $\delta_{\text{H}}$  1.44 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 2.21 (3H, s,  $\text{CH}_3$ ), 4.49 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 7.38 (1H, dd, H-6), 7.77 (1H, d, H-7), 8.33 (1H, d, H-4), 10.37 (1H, s,  $\text{NH-CH}_2$ ), 12.22 (1H, bs,  $\text{NH/OH}$ ),  $J_{4,6}=1.2$ ,  $J_{6,7}=8.8$ ,  $J_{\text{CH}_2,\text{CH}_3}=7.2$ ,  $\delta_{\text{C}}$  (75 MHz) 14.1 ( $\text{CH}_2\text{CH}_3$ ), 24.1 ( $\text{CH}_3\text{CO}$ ), 61.5 ( $\text{CH}_2\text{CH}_3$ ), 99.2 (C-4), 116.9 (C-7), 121.3 (C-6), 133.2 (C-5 and -7a), 137.3 (C-2), 137.5 (C-3a), 158.0 ( $\text{CH}_3\text{CO}$ ), 168.7 ( $\text{C=O}$ ).

Ethyl 6-acetamido-1-(3-hydroxypropoxy)benzimidazole-2-carboxylate (120)

Ethyl 5-acetamidobenzimidazole-2-carboxylate 3-oxide (119) (0.5g) was dissolved in the minimum of anhydrous DMF (20ml) and potassium carbonate (0.3g) added resulting in a precipitate forming. 3-Bromopropan-1-ol (104) (0.3g) was then added to the mixture and stirring maintained under a calcium chloride guard for 24h, the precipitate redissolving over time. The reaction mixture was then poured into water (75ml) and extracted with dichloromethane (3x25ml), the organic layers were combined, washed with water (100ml) and dried over magnesium sulphate, and the solvent was removed *in vacuo* to yield a dark oil which crystallised on standing. The solid was recrystallised from toluene: yield 0.08g (13%), m.p. 161-163°C, (Found: C, 56.2; H, 6.0; N, 13.1.  $\text{C}_{15}\text{H}_{18}\text{N}_3\text{O}_5$  requires C, 56.2; H, 5.7; N, 13.1%),  $\delta_{\text{H}}$  1.34 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 1.94 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 2.11 (3H, s,  $\text{CH}_3$ ), 3.65 (2H, m,  $\text{CH}_2\text{-OH}$ ), 4.37-4.50 (4H, m,  $\text{CH}_2\text{-ON}$  and  $\text{CH}_2\text{CH}_3$ ), 4.65 (1H, bs,



CH<sub>2</sub>-OH), 7.36 (1H, dd, H-5), 7.75 (1H, d, H-4), 8.19 (1H, d, H-7), 10.28 (1H, s, NH),  $\delta_C$  14.0 (CH<sub>2</sub>CH<sub>3</sub>), 24.0 (CH<sub>3</sub>), 31.2 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 57.3 (HO-CH<sub>2</sub>), 61.4 (CH<sub>2</sub>CH<sub>3</sub>), 77.2 (CH<sub>2</sub>-O-N), 98.7 (C-7), 117.4 (C-4), 121.7 (C-5), 131.6 (C-6), 132.9 (C-3a), 136.6 (C-7a), 137.8 (C-2), 168.2 (CH<sub>3</sub>CO), 168.6 (C=O).

The above procedure was repeated at both 0°C and 100°C in an attempt to improve the yield of the reaction while monitoring its progress by tlc. The product was isolated in both cases but the best yield obtained was 30% in the reaction at room temperature.

A second change to the procedure was the replacement of 3-bromopropan-1-ol (104) with 3-iodopropan-1-ol (121) but again no improvement in yield resulted.

#### 3-Iodopropan-1-ol (121)

3-Bromopropan-1-ol (104) (5.0g) and sodium iodide (5.5g) were heated under reflux in anhydrous acetone (50ml) for 17h. The reaction mixture was allowed to cool before the removal of the acetone *in vacuo*. The resultant oil was then dissolved in dichloromethane (50ml), washed with water (2x50ml) and then with sodium thiosulphate until the organic layer was decolourised. The organic layer was then dried over magnesium sulphate. Distillation at high vacuum yielded a colourless oil: Yield 1.5g (22%), b.p. 30°C / 0.1mmHg, (lit.<sup>127</sup> 88°C),  $\delta_H$  2.03 (2H, quin., CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 3.29 (2H, q, CH<sub>2</sub>-OH), 3.42 (1H, bs, CH<sub>2</sub>-OH), 3.68 (2H, bs, CH<sub>2</sub>-I),  $\delta_C$  4.0 (CH<sub>2</sub>-I), 36.1 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 62.5 (CH<sub>2</sub>-OH).

#### 5-Acetamido-2-cyanobenzimidazole 3-oxide (123)

4-Acetamido-N-cyanomethyl-2-nitroaniline (122) (4.0g) and potassium carbonate (3.2g) were heated together in ethanol (250ml) under reflux for 2h. The solvent was evaporated off and the resultant solid dissolved in water (200ml) and acidified to pH 1 with concentrated hydrochloric acid. The precipitate was collected and recrystallised from water and ethanol: yield 1.15g (31%), m.p. 234-238°C (lit.<sup>87</sup> 233-234°C),  $\delta_H$  2.12 (3H, s, CH<sub>3</sub>), 7.31 (1H, dd, H-6), 7.69 (1H, d, H-7), 8.27 (1H, d, H-4), 10.30 (1H, bs, NH-CH<sub>2</sub>),  $J_{4,6}=1.2$ ,

4-Acetamido-*N*-cyanomethyl-2-nitroaniline (122) (4.0g) and potassium carbonate (3.2g) were heated together in ethanol (250ml) under reflux for 2h. The solvent was evaporated off and the resultant solid dissolved in water (200ml) and acidified to pH 1 with concentrated hydrochloric acid. The precipitate was collected and recrystallised from water and ethanol: yield 1.15g (31%), m.p. 234-238°C (lit.<sup>87</sup> 233-234°C),  $\delta_{\text{H}}$  2.12 (3H, s, CH<sub>3</sub>), 7.31 (1H, dd, H-6), 7.69 (1H, d, H-7), 8.27 (1H, d, H-4), 10.30 (1H, bs, NH-CH<sub>2</sub>),  $J_{4,6}=1.2$ ,  $J_{6,7}=8.8$ ,  $J_{\text{CH}_2, \text{CH}_3}=7.2$ ,  $\delta_{\text{C}}$  (75 MHz) 24.4 (CH<sub>3</sub>CO), 98.4 (CN), 111.1 (C-5), 117.7 (C-7), 121.6 (C-4), 131.8 (C-3a), 134.5 (C-7a), 138.4 (C-6) and (C-2), 169.0 (CH<sub>3</sub>CO).

6-Acetamido-2-cyano-1-(3-hydroxypropoxy)benzimidazole (124)

5-acetamido-2-cyano benzimidazole-3-oxide (123) (0.4g) was dissolved in the minimum of anhydrous DMF (20ml) and potassium carbonate (0.3g) added. 3-Bromopropan-1-ol (104) (0.3g) was then added to the mixture and stirring maintained under a calcium chloride guard for 24h. The reaction mixture was then poured into water (75ml) and extracted with dichloromethane (3x25ml), the organic layers were combined, washed with water (100ml) and dried over magnesium sulphate. The solvent was removed *in vacuo* and the solid recrystallised from water and ethanol: yield 0.41g (81%), m.p. 166-167°C, (Found: C, 51.6; H, 5.6; N, 18.6. C<sub>13</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>·1.5H<sub>2</sub>O requires C, 51.8; H, 5.2; N, 18.6%),  $\delta_{\text{H}}$  1.97 (2H, quin., CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 2.10 (3H, s, CH<sub>3</sub>), 3.61 (2H, q, CH<sub>2</sub>-OH), 4.60 (2H, t, CH<sub>2</sub>-ON), 4.74 (1H, bs, CH<sub>2</sub>-OH), 7.42 (1H, d, H-5), 7.77 (1H, d, H-4), 8.24 (1H, s, H-7), 10.34 (1H, s, NH),  $\delta_{\text{C}}$  24.1 (CH<sub>3</sub>), 31.2 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 57.0 (HO-CH<sub>2</sub>), 78.5 (CH<sub>2</sub>-O-N), 98.3 (C-7), 110.3 (C-3a), 118.0 (C-4), 121.7 and 121.8 (CN and C-5), 130.2 (C-6), 134.1 (C-7a), 138.8 (C-2), 168.8 (C=O).

## Chapter 3

### Experimental

#### *N*-(4,5-Dichloro-2-nitrophenyl)glycine ethyl ester (129)

1,2-Dichloro-4-fluoro-5-nitrobenzene (133) (25.1g), glycine ethyl ester hydrochloride (17.0g) and sodium bicarbonate (19.5g) were heated under reflux in ethanol (450ml) for 4h. The reaction was poured into ice/water (200ml), and the resultant precipitate was collected and recrystallised from ethanol to give yellow needles: yield 29.2g (83%), m.p. 119-120°C (lit.<sup>117</sup> 122°C),  $\delta_{\text{H}}$  1.25 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 4.20 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 4.31 (2H, d,  $\text{CH}_2\text{-NH}$ ), 7.29 (1H, s, H-6), 8.25 (1H, s, H-3), 8.43 (1H, t,  $\text{NH-CH}_2$ ),  $J_{\text{CH}_2,\text{CH}_3}=7.1$ ,  $J_{\text{NH,CH}}=5.8$ ,  $\delta_{\text{C}}$  14.0 ( $\text{CH}_2\text{CH}_3$ ), 44.5 ( $\text{NH-CH}_2$ ), 61.1 ( $\text{CH}_2\text{CH}_3$ ), 116.5 (C-6), 117.4 (C-4), 127.0 (C-3), 130.6 (C-2), 139.5 (C-5), 143.6 (C-1), 169.4 (C=O),  $m/z = 292 / 294 / 296$  ( $\text{M}^+$ ).

#### Ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134)

*N*-(4,5-Dichloro-2-nitrophenyl)glycine ethyl ester (129) (27.1g) and potassium carbonate (12.9g) were heated under reflux in ethanol (300 ml) for 3h. The reaction mixture was partitioned between dichloromethane (250ml) and water (250ml), the organic phase being extracted with water (500ml). The combined water layers were extracted with dichloromethane (250ml) and then acidified with concentrated hydrochloric acid. The yellow precipitate formed was collected and recrystallised from ethanol: yield 9.0g (35%), m.p. 212-220°C (decomp.) (lit.<sup>98</sup> 218-221°C),  $\delta_{\text{H}}$  1.38 (1H, t,  $\text{CH}_2\text{CH}_3$ ), 4.44 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 7.91 and 8.12 (2H, 2s, H-4 and H-7),  $J_{\text{CH}_2,\text{CH}_3}=7.0$ ,  $\delta_{\text{C}}$  (75 MHz) 14.0 ( $\text{CH}_2\text{CH}_3$ ), 61.9 ( $\text{CH}_2\text{CH}_3$ ), 111.7 (C-7), 122.4 (C-4), 126.2 (C-5), 128.2 (C-6), 132.1 (C-3a), 136.1 (C-7a), 139.7 (C-2), 157.5 (C=O),  $m/z = 274 / 276 / 278$  ( $\text{M}^+$ ).

Production of a mixture of ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134) and 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (135)

*N*-(4,5-Dichloro-2-nitrophenyl)glycine ethyl ester (129) (20.6g) and potassium carbonate (10.0g) were heated under reflux in ethanol (250 ml) for 4h and the mixture left to cool overnight and then worked up following the above procedure for (134). On analysis of the crude product by  $^1\text{H}$  nmr it was found that the material isolated was a mixture of the desired product (134) and its decarboxylated counterpart (135). Rather than try to separate the mixture it was taken directly through to the hydrochloride by refluxing in concentrated hydrochloric acid (see procedure below).

5,6-Dichlorobenzimidazole 3-oxide hydrochloride (135a) from a mixture of compounds (134) and (135)

A mixture of crude ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134) and 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (135) was heated under reflux in concentrated hydrochloric acid (450ml) for 3h. The reaction mixture was concentrated *in vacuo* and allowed to cool, crystallisation occurring. The product was collected by filtration, and a second crop of crystals obtained from the filtrate: yield 8.3g (49%, over the cyclisation and hydrolysis/decarboxylation stages), m.p.212-214°C (decomp.) (lit.<sup>98</sup> 204-207°C, decomp.).

5,6-Dichlorobenzimidazole 3-oxide hydrochloride (128a)

Ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (127) (5.0g) was heated under reflux in concentrated hydrochloric acid (125ml) for 5h; after cooling a precipitate had formed which was collected and recrystallised from concentrated hydrochloric acid to yield opaque needles: yield 2.0g (46%), m.p.212-214°C (lit.<sup>98</sup> 204-204°C, decomp.),  $\delta_{\text{H}}$  8.04 and 8.12 (2H, 2s, H-4 and H-7), 9.25 (1H, s, H-2),  $\delta_{\text{C}}$  (75 MHz) 109.7 (C-5), 113.0 (C-4), 117.8 (C-7), 128.4 (C-3a), 129.9 (C-6), 130.5 (C-7a), 139.8 (C-2).

### 5,6-Dichlorobenzimidazole 3-oxide (135)

The 5,6-dichlorobenzimidazole 3-oxide hydrochloride (135a) (2.0g) was dissolved in ammonia solution (80ml), stirred for 0.5h then concentrated *in vacuo* to ca. 40ml. The resultant precipitate was collected by filtration and recrystallised from ethanol/water: yield 1.5g (41%), m.p. 200-210°C (lit.<sup>98</sup> 224-226°C),  $\delta_H$  7.72 and 8.00 (2H, 2s, H-4 and H-7), 8.50 (1H, s, H-2),  $\delta_C$  (75 MHz) 111.1 (C-4), 121.6 (C-7), 124.7 (C-6), 125.8 (C-5), 131.4 (C-3a), 139.1 (C-7a), 142.8 (C-2).

### 5,6-Dichloro-1-(3-hydroxypropoxy)benzimidazole (136)

i) with sodium ethoxide as the base

To a solution of 5,6-dichlorobenzimidazole 3-oxide (135) (0.3g) in ethanol (30ml), a solution of sodium ethoxide (1.2ml) was added, [sodium (0.6g) dissolved in ethanol (20ml)] with stirring. After 0.5h the reaction was evaporated to dryness and the white solid dissolved in the minimum of DMF (10ml). 3-Bromopropan-1-ol (0.2g) was then added and stirring maintained for 24h. The reaction mixture was poured into water (50ml) and extracted with dichloromethane (3x25ml). The organic extracts were combined, washed with a dilute solution of sodium hydroxide (0.1M, 10ml) and then twice with water (2x75ml) followed by drying over magnesium sulphate. The solvent was removed *in vacuo* and the white solid obtained was recrystallised from toluene: yield 0.10g (31%), m.p. 70-72°C. (Found: C, 44.5; H, 4.1; N, 10.3.  $C_{10}H_{10}Cl_2N_2O_2 \cdot 0.5H_2O$  requires C, 44.5; H, 3.7; N, 10.4%).  $\delta_H$  1.94 (2H, quin.,  $CH_2-CH_2-CH_2$ ), 3.62 (2H, q,  $HO-CH_2$ ), 4.49 (2H, t,  $CH_2-ON$ ), 4.75 (1H, t,  $HO-CH_2$ ), 8.00 (2H, s, H-4 and H-7), 8.71 (1H, s, H-2),  $J_{CH_2,CH_2}=6.2$ ,  $J_{CH,OH}=5.2$ ,  $\delta_C$  30.8 ( $CH_2-CH_2-CH_2$ ), 56.9 ( $CH_2-OH$ ), 77.4 (N-O- $CH_2$ ), 110.7 (C-7), 121.5 (C-4), 125.1 (C-6), 126.2 (C-5), 129.3 (C-3a), 138.5 (C-7a), 142.0 (C-2).

ii) with potassium carbonate as the base

5,6-Dichlorobenzimidazole 3-oxide (135) (0.5g) was dissolved in the minimum of dried DMF (5ml), potassium carbonate (0.34g) was then added followed by

3-bromopropan-1-ol (0.34g) and stirring continued for 18h. The reaction mixture was poured into water (20ml) and extracted with dichloromethane (3x20ml), the organic extracts were combined, washed with water (2x50ml), dried over magnesium sulphate and concentrated to a pale yellow liquid which crystallised in the freezer over a few days and was recrystallised twice from toluene, once with charcoal: yield 0.26g (39%), m.p.69-71°C.

iii) with triethylamine as the base

5,6-Dichlorobenzimidazole 3-oxide (135) (0.5g) was dissolved in ethanol (25ml) along with triethylamine (0.27g) and 3-bromopropan-1-ol (0.38g). The reaction mixture was heated under reflux for 3h with a further aliquot of triethylamine (0.14g) added after 2h. After cooling a crude product was isolated from the reaction mixture and recrystallised from ethanol/toluene: yield 0.1g (16%), m.p.69-70°C.

Ethyl 5,6-dichloro-1-(3-hydroxypropoxy)benzimidazole-2-carboxylate (137)

Ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134) (0.25g) was dissolved in the minimum of DMF (20ml); addition of potassium carbonate (0.14g), led to the formation of a precipitate. This was dissolved by the addition of further DMF (5ml) followed by 3-bromopropan-1-ol (0.13g). The mixture was stirred overnight and then poured into water (50ml) and extracted with dichloromethane (3x50 ml). The organic layers were combined and washed twice with equal volumes of water (2x100ml) then dried over magnesium sulphate. The solution was concentrated to an oil which crystallised upon cooling; the solid was recrystallised from ethanol yielding pale needle-like crystals: yield 0.13g (43%), m.p.116-118°C. (Found: C, 47.3; H, 4.5; N, 8.2.  $C_{13}H_{14}Cl_2N_2O_4$  requires C, 46.9; H, 4.2; N, 8.4%).  $\delta_H$  1.39 (3H, t,  $CH_2CH_3$ ), 2.10 (2H, quin.,  $CH_2-CH_2-CH_2$ ), 3.65 (2H, q,  $CH_2-OH$ ), 4.40-4.55 (4H, m,  $CH_2-ON$ ,  $CH_2CH_3$ ), 4.73 (1H, t,  $CH_2-OH$ ), 8.12 and 8.17 (2H, 2s, H-4 and H-7),  $J_{CH_2,CH_3}=7.3$ ,  $\delta_C$  14.1 ( $CH_2CH_3$ ), 31.0 ( $CH_2-CH_2-CH_2$ ), 57.2 ( $CH_2CH_3$ ), 62.4 ( $CH_2-OH$ ), 77.6 (N-O- $CH_2$ ), 112.1 (C-4), 123.0 (C-7), 127.0 (C-5), 129.1 (C-6), 130.9 (C-3a), 136.2 (C-2), 139.5 (C-7a), 157.1 (C=O),  $m/z$  =332 / 334/ 336 ( $M^{+}$ ).



The reaction was also repeated at low temperature following the above reaction procedure. The ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (134) (0.25g) was dissolved in DMF (20ml) and the solution cooled to  $-30^{\circ}\text{C}$  before the addition of the potassium carbonate (0.14g) and 3-bromopropan-1-ol (0.13g). After 4h at  $-30^{\circ}\text{C}$  the reaction mixture was allowed to heat up gradually to  $0^{\circ}\text{C}$  and was then held at that temperature for 2h followed by a further 2h at room temperature. The correct product was isolated and characterised as above but was in a rather low yield: yield 0.1g (17%), m.p.  $115-117^{\circ}\text{C}$ .

The reaction to produce (137) was also repeated with heating at  $90^{\circ}\text{C}$ . Ethyl 5,6-dichlorobenzimidazole-2-carboxylate 3-oxide (135) (2.0g) was dissolved in the minimum of DMF (40ml), followed by the addition of potassium carbonate (1.1g) and stirring for 0.5h. 3-Bromopropan-1-ol (1.1g) was then added and the reaction heated to  $90^{\circ}\text{C}$  and held at that temperature for 6h. The reaction was worked up as previously, whereupon a mixture of products was isolated and found by  $^1\text{H}$  nmr to consist of 90% of the decarboxylated compound (136) and 10% of the desired compound (137). Separation of the mixture of products was not attempted, but the mixture was hydrolysed/decarboxylated in concentrated hydrochloric acid (see below).

Hydrolysis of a mixture of ethyl 5,6-dichloro-1-(3-hydroxypropoxy)benzimidazole-2-carboxylate (137) and 5,6-dichloro-1-(3-hydroxypropoxy)benzimidazole (136)

A mixture of ethyl 5,6-dichloro-1-(3-hydroxypropoxy)benzimidazole-2-carboxylate (137) (10%) and 5,6-dichloro-1-(3-hydroxypropoxy)benzimidazole (136) (90%) (0.75g) was heated under reflux in concentrated hydrochloric acid (40ml) for 3h. On cooling, a solid precipitated from the reaction mixture and was recrystallised from ethanol/toluene: yield 0.30g (16%), m.p.  $67-68^{\circ}\text{C}$ ,  $\delta_{\text{H}}$  1.90 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.63 (2H, t,  $\text{CH}_2\text{-OH}$ ), 4.46 (2H, t,  $\text{N-O-CH}_2$ ), 4.79 (1H, bs,  $\text{CH}_2\text{-OH}$ ), 7.98 (2H, s, H-4 and H-7), 8.72 (1H, s, H-2),  $\delta_{\text{C}}$  31.0 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 57.1 ( $\text{CH}_2\text{-OH}$ ), 77.5 ( $\text{N-O-CH}_2$ ), 111.0 (C-7), 121.2 (C-4), 125.0 (C-6), 125.9 (C-5), 129.4 (C-3a), 138.5 (C-7a), 142.2 (C-2).

Attempted reaction of pentachloronitrobenzene and glycine ethyl ester hydrochloride

Pentachloronitrobenzene (138) (1.1g), glycine ethyl ester hydrochloride (0.6g) and sodium bicarbonate (0.7g) were heated under reflux in ethanol (30ml) and toluene (30ml) for 4h. Tlc and  $^1\text{H}$  nmr analysis of the crude reaction mixture showed no reaction to have occurred.

Attempted synthesis of N-(*o*-nitrophenyl)glycine methyl ester

To a solution of *o*-fluoronitrobenzene (139) (1.0g) in methanol (20ml), glycine methyl ester hydrochloride (0.9g) and sodium bicarbonate (1.2g) were added and the mixture heated under reflux for 3h. The reaction mixture was concentrated *in vacuo*, and partitioned between dichloromethane (50ml) and water (50ml). The aqueous layer was extracted with dichloromethane (3x25ml) and the organic layers combined, washed with water and dried over magnesium sulphate. The solvent was removed to yield a yellow oil which upon analysis was found to be a complex mixture of products comprising mainly of the starting material (139).

The separation of the mixture was not attempted as an easier method was available for the synthesis of *o*-nitrophenylglycine ethyl ester (141).

N-(*o*-Nitrophenyl)glycine (140)

A solution of *o*-fluoronitrobenzene (139) (5.0g) in ethanol (130ml) was added to a solution of glycine (2.7g) and sodium bicarbonate (11.9g) in water (80ml) with stirring, a white precipitate forming. The reaction mixture was then heated under reflux for 8h and allowed to cool. The mixture was concentrated *in vacuo*, and partitioned between ether (100ml) and water (100ml). The aqueous layer was then acidified with concentrated hydrochloric acid to yield an orange/yellow precipitate which was recrystallised from ethanol: yield 2.9g (50%), m.p. 188-190°C (lit.<sup>129</sup> 199°C),  $\nu_{\text{max}}$  3375 (N-H), 1718 (C=O), 1505, 1357 and 1320  $\text{cm}^{-1}$  ( $\text{NO}_2$ ),  $\delta_{\text{H}}$  4.20 (2H, d,  $\text{NH}-\text{CH}_2$ ), 6.75 (1H, t, H-5), 6.95 (1H, d, H-6), 7.55 (1H, t, H-4), 8.10 (1H, d, H-3), 8.40 (1H, t,  $\text{NH}-\text{CH}_2$ ),  $J_{3,4}=10.0$ ,  $J_{3,5}=4.9$ ,  $J_{\text{NH}-\text{CH}_2}=5.2$ ,  $\delta_{\text{C}}$  44.2 ( $\text{NH}-\text{CH}_2$ ), 114.9 (C-5), 115.7 (C-4), 126.0 (C-3), 131.5 (C-1), 136.4 (C-6), 144.4 (C-2), 170.9 (C=O).



N-(*o*-Nitrophenyl)glycine ethyl ester (141)

N-(*o*-Nitrophenyl)glycine (140) (2.7g) was dissolved in ethanol (250ml) and the solution saturated with hydrogen chloride gas over 1.5h. The acidified solution was then heated under reflux for 8h and allowed to cool. The solvent was evaporated off to yield an orange/yellow solid which was recrystallised from propan-2-ol alcohol yielding a yellow crystalline solid: yield 1.80g (60%), m.p.77-79°C (lit.<sup>130</sup> 80°C),  $\delta_{\text{H}}$  1.25 (3H, t, CH<sub>2</sub>CH<sub>3</sub>), 4.20 (2H, q, CH<sub>2</sub>CH<sub>3</sub>), 4.30 (2H, d, CH<sub>2</sub>-NH), 6.75 (1H, t, H-5), 6.95 (1H, d, H-6), 7.55 (1H, t, H-4), 8.15 (1H, d, H-3), 8.40 (1H, bs, NH-CH<sub>2</sub>),  $J_{3,4}=10.0$ ,  $J_{3,5}=2.6$ ,  $J_{\text{NH-CH}_2}=5.1$ ,  $\delta_{\text{C}}$  14.6 (CH<sub>2</sub>CH<sub>3</sub>), 44.9 (CH<sub>2</sub>CH<sub>3</sub>), 115.4 (C-5), 116.5 (C-4), 126.6 (C-3), 132.0 (C-1), 137.0 (C-6), 145.0 (C-2), 170.3 (C=O).

Synthesis of a mixture of N-(*o*-nitrophenyl)glycine (140) and N-(*o*-nitrophenyl)glycine ethyl ester (141)

A solution of *o*-fluoronitrobenzene (139) (15.0g) in ethanol (390ml) was added to a solution of glycine (8.0g) and sodium bicarbonate (35.7g) in water (240ml) with stirring, a white precipitate forming. The reaction mixture was then heated under reflux for 11h and allowed to cool before the work-up procedure was followed as above. Two crops of crystals were isolated from the recrystallisation solvent, ethanol. Crop 1: yield 10.0g (57%), m.p.176-178°C (lit.<sup>129</sup> 199°C) was identified as compound (140). Crop 2: yield 3.8g (16%), m.p.78-80°C (lit.<sup>130</sup> 80°C) was identified as compound (141).

Ethyl benzimidazole-2-carboxylate 3-oxide (142)

N-(*o*-Nitrophenyl)glycine ethyl ester (141) (6.0g) was dissolved in ethanol (200ml) and heated under reflux with potassium carbonate (4.1g) for 2h then allowed to cool. The reaction mixture was concentrated *in vacuo* then partitioned between dichloromethane (150ml) and water (150ml). The aqueous layer was then acidified with concentrated hydrochloric acid, no precipitate forming but gas being evolved; concentration *in vacuo* then led to a yellow solid product which was recrystallised from ethanol/toluene: yield 3.5g (63%), m.p.166-167°C (lit.<sup>131</sup> 166-167°C),  $\delta_{\text{H}}$  1.40 (3H, t, CH<sub>2</sub>CH<sub>3</sub>), 4.45 (2H, q, CH<sub>2</sub>CH<sub>3</sub>), 7.50 (2H, m, H-5

and H-6), 7.82 (2H, m, H-4 and H-7),  $J_{4,6}=5.8$ ,  $J_{5,6}=5.8$ ,  $\delta_C$  14.0 ( $\text{CH}_2\text{CH}_3$ ), 62.6 ( $\text{CH}_2\text{CH}_3$ ), 111.8 (C-7), 118.5 (C-4), 125.9 (C-5), 126.5 (C-6), 132.0 (C-7a), 132.8 (C-3a), 135.9 (C-2), 156.1 (C=O).

Synthesis of a mixture of ethyl benzimidazole-2-carboxylate 3-oxide (142) and benzimidazole 3-oxide (143)

N-(*o*-Nitrophenylglycine ethyl ester (141) (8.7g) was dissolved in ethanol (150ml) and the solution heated under reflux with potassium carbonate (5.8g) for 2h then allowed to cool. The reaction mixture was concentrated *in vacuo* and the residue then partitioned between dichloromethane (175ml) and water (175ml). The aqueous layer was then acidified with concentrated hydrochloric acid, no precipitate forming but gas being evolved; and concentration *in vacuo* gave a yellow solid product.  $^1\text{H}$  nmr analysis of the crude product showed it to be a mixture of the desired product (134) and its decarboxylated counterpart (135): yield 13.0g (>100%), m.p.160-164°C, the ratio of (142) : (143) was approximately 2.5 : 1.

Benzimidazole 3-oxide hydrochloride (143a)

The above mixture of ethyl benzimidazole-2-carboxylate 3-oxide (142) and benzimidazole 3-oxide (143) (12.9g) was heated under reflux in concentrated hydrochloric acid (325 ml) for 2 h, then allowed to cool. The reaction mixture was concentrated *in vacuo* to an orange solid which was recrystallised from propan-2-ol: yield 4.0g (40% based on the ester), m.p.206-208°C (lit.<sup>73</sup> 199-200°C),  $\delta_H$  (300MHz) 7.62-7.71 (2H, m, H-5,6), 7.89-7.96 (2H, m, H-4,7), 10.03 (1H, s, H-2),  $J_{4,5}=J_{5,6}=7.6$ ,  $\delta_C$  111.9 (C-7), 114.9 (C-4), 126.1 (C-5), 126.7 (C-6), 128.5 (C-3a), 129.1 (C-7a), 136.7 (C-2).

Benzimidazole 3-oxide (143)

Benzimidazole 3-oxide hydrochloride (143a) (1.5g) was dissolved in ammonia solution (60ml) and stirred for 0.5h. The solution was concentrated *in vacuo* to aid precipitation. The product was isolated by filtration and recrystallised from ethanol: yield 0.70g (85%), m.p.211-213°C, (lit.<sup>73</sup> 214-216°C),  $\delta_H$  7.15 and 7.25 (2H, 2t, H-5 and H-6), 7.50

and 7.61 (2H, 2d, H-4 and H-7), 8.38 (1H, s, H-2),  $J_{4,5}=J_{5,6}=7.8$ ,  $\delta_C$  109.5 (C-7), 119.9 (C-5), 121.9 (C-6), 122.9 (C-4), 131.9 (C-3a), 139.3 (C-2), 140.0 (C-7a).

1-[(3-Tetrahydropyran-2-yloxy)propoxy]benzimidazole (136)

Benzimidazole 3-oxide (143) (0.5g) was dissolved in ethanol (10ml) and sodium ethoxide solution (3.2ml) added [sodium (0.65g) dissolved in ethanol (20ml)] with stirring. After 0.5h, concentration *in vacuo* led to a white solid. This solid was then dissolved in the minimum of DMF (10ml) and the solution stirred for 24h at room temperature with the THP ether (105) (1.0g). The reaction mixture was poured into water (100ml) and extracted with dichloromethane (3x75ml), the organic layers were combined, washed with sodium hydroxide (0.1M, 75ml), followed by water (2x100ml) then dried over magnesium sulphate. The solvent was removed under reduced pressure and the purification of crude product attempted on a column of silica. The gummy product could not be purified completely by column chromatography and proved too thermally unstable to be distilled at low pressure.  $^1H$  nmr showed the correct resonances to be present but with incorrect integration; the product was therefore taken through to the next synthetic stage in an impure form. Yield 0.5g (48%),  $\delta_H$  1.36 -1.81 (6H, m, H-2', H-3' and H-4.), 2.13 (2H, quin.,  $CH_2-CH_2-CH_2$ ), 3.31-3.93 (4H, m,  $CH_2-OTHP$  and H-5'), 4.46 (2H, t, N-O- $CH_2$ ), 4.53 (1H, s, H-1'), 7.24-7.41 (2H, 2t, H-5 and H-6), 7.62 (1H, d, H-4), 7.75 (1H, d, H-7), 8.58 (1H, s, H-2),  $J_{4,5}=7.5$ ,  $J_{CH_2,CH_3}=7.0$ .

Ethyl 1-[(3-tetrahydropyran-2-yloxy)propoxy]benzimidazole-2-carboxylate (146)

Ethyl benzimidazole-2-carboxylate 3-oxide (142) (0.50g) was dissolved in DMF (20ml) and potassium carbonate (0.74g) added. The resultant mixture was stirred for 1h at room temperature before the addition of the THP ether (105) (0.60g), and stirring was then continued for a further 24h. The reaction mixture was then poured into water (100ml) and extracted with dichloromethane (3x50ml). The organic layers were combined and washed with water (150ml), dried over magnesium sulphate and concentrated to a gum which was purified on a column of silica. Yield 0.04g (5%),  $\delta_H$  1.35 (3H, t,  $CH_2CH_3$ ), 1.46 -1.75 (6H, m, H-2',

H-3' and H-4'), 2.10 (2H, quin., CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 3.43-3.91 (4H, m, CH<sub>2</sub>-OTHP and H-5'), 4.40 (2H, q, CH<sub>2</sub>CH<sub>3</sub>), 4.50 (2H, m, N-O-CH<sub>2</sub>), 4.63 (1H, s, H-a), 7.36 (1H, t, H-6), 7.48 (1H, t, H-5), 7.70 (1H, d, H-4), 7.81 (1H, d, H-7),  $J_{4,5}=8.2$ ,  $J_{CH_2,CH_3}=7.0$ ,  $\delta_C$  14.1 (CH<sub>2</sub>-CH<sub>3</sub>), 19.8 (C-2'), 25.5 (C-4'), 28.6 (C-3'), 30.8 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 62.0 (CH<sub>2</sub>CH<sub>3</sub>), 62.3 (CH<sub>2</sub>-OTHP), 63.6 (C-5'), 77.3 (N-O-CH<sub>2</sub>), 98.7 (C-1'), 110.5 (C-7), 122.0 (C-4), 124.5 (C-6), 126.6 (C-5), 131.8 (C-3a), 137.3 (C-2), 137.4 (C-7a), 157.9 (C=O).

1-(3-Hydroxypropoxy)benzimidazole (145) from the reaction of (144) with pyridinium *p*-toluenesulphonate (PPTS)

(For the synthesis of PPTS see chapter 2 experimental, p106)

1-[(3-Tetrahydropyran-2-yloxy)propoxy]benzimidazole (144) (0.2g) was dissolved in ethanol (5ml) and PPTS (0.02g) added with stirring and heating to *ca.* 55°C. The temperature and stirring were maintained for 5h. The reaction mixture was concentrated *in vacuo* and purification attempted on a column of silica, but the product was found to be extremely polar and difficult to elute from the column, hence the gum isolated was not a completely pure product. Like compound (144), (145) was also found to be too thermally unstable to be distilled at low pressure. The data shown relates to the impure product: yield 0.05g (38%),  $\delta_H$  1.95 (2H, quin., CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 3.64 (2H, t, CH<sub>2</sub>-OH), 4.56 (2H, t, N-O-CH<sub>2</sub>), 7.17 (1H, d, H-4), 7.56 (2H, d, H-5 and H-6), 7.86 (1H, d, H-7), 9.45 (1H, s, H-2),  $J_{4,5}=J_{5,6}=7.5$ ,  $J_{CH_2,CH_3}=6.5$ ,  $\delta_C$  30.8 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 57.0 (CH<sub>2</sub>-OH), 78.1 (N-O-CH<sub>2</sub>), 111.0 (C-3a), 116.2 (C-7a), 125.8 (C-4 and C-7), 127.1 (C-5 and C-6), 128.6 (C-2).

1-(3-Hydroxypropoxy)benzimidazole (145)

Benzimidazole 3-oxide (143) (0.1g) was dissolved in sodium ethoxide solution (4.0ml) [sodium (0.1g) dissolved in ethanol (20ml)] and stirred for 0.5h. The solution, when concentrated under reduced pressure, yielded a solid which was then dissolved in DMF (10ml) and stirred for 24h at room temperature with 3-bromopropan-1-ol (0.1g). The reaction mixture was then poured into water (100ml) and extracted with dichloromethane (3x75ml). The organic

layers were combined and washed with sodium hydroxide (0.1M, 75ml) then water (2x100ml), dried over magnesium sulphate and concentrated to a viscous oil.  $\delta_{\text{H}}$  1.93 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.69 (2H, q,  $\text{HO-CH}_2$ ), 4.48 (2H; t,  $\text{CH}_2\text{-O-N}$ ), 4.86 (1H, t,  $\text{CH}_2\text{-OH}$ ), 7.33 (2H, 2t, H-5,6), 7.63 (1H, d, H-4), 7.74 (1H, d, H-7), 8.61 (1H, s, H-2),  $J_{4,5}=7.5$ ,  $J_{\text{CH}_2,\text{CH}_3}=6.3$ ,  $J_{\text{CH}_2,\text{OH}}=5.0$ . Attempts to purify the product as above also failed and the attempted reaction at 75°C resulted in no detection of any discernable product presumably due to the thermal instability of the product.

The above reaction was repeated with potassium carbonate as the base, following the same procedure. Although the product (145) was furnished in a slightly greater yield, (145) was never isolated in a pure enough form to undergo C/H/N analysis.

Ethyl 1-(3-hydroxypropoxy)benzimidazole-2-carboxylate (147)

Ethyl benzimidazole-2-carboxylate 3-oxide (147) (1.0g) was dissolved in DMF (20ml) and potassium carbonate (0.74g) added. The resultant mixture was stirred for 0.5h at room temperature, 3-bromopropan-1-ol (0.74g) was added and stirring maintained for a further 24h. The reaction mixture was then poured into water (100ml) and extracted with dichloromethane (3x50ml). The organic layers were combined, washed with water (150ml), dried over magnesium sulphate and concentrated to give an oil which crystallised on standing. The solid collected was purified on a column of silica and recrystallised from toluene/ethanol: yield 0.10g (8%), m.p. 92-94°C. (Found: C, 58.7; H, 6.2; N, 10.3.  $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_4$  requires C, 59.1; H, 6.1; N, 10.6%).  $\delta_{\text{H}}$  1.36 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 1.99 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.67 (2H, q,  $\text{HO-CH}_2$ ), 4.39-4.55 (4H, m,  $\text{CH}_2\text{CH}_3$  and  $\text{CH}_2\text{-O-N}$ ), 4.86 (1H, t,  $\text{CH}_2\text{-OH}$ ), 7.37-7.56 (2H, 2t, H-5 and H-6), 7.71-7.85 (2H, 2d, H-4 and H-7),  $J_{4,5}=7.0$ ,  $J_{4,6}=1.2$ ,  $J_{\text{CH}_2,\text{CH}_3}=7.2$ ,  $J_{\text{CH}_2,\text{CH}_2}=6.4$ ,  $\delta_{\text{C}}$  14.5 ( $\text{CH}_2\text{CH}_3$ ), 31.5 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 57.5 ( $\text{CH}_2\text{-OH}$ ), 62.3 ( $\text{CH}_2\text{CH}_3$ ), 77.5 (N-O- $\text{CH}_2$ ), 110.6 (C-4), 122.0 (C-7), 124.5 (C-6), 126.6 (C-5), 131.8 (C-3a), 137.4 (C-7a), 137.2 (C-2), 157.9 (C=O).



2-Cyano-5-fluorobenzimidazole 3-oxide (149)

*N*-Cyanomethyl-4-fluoro-2-nitroaniline (4.0g) (148) was dissolved in hot ethanol (250ml) before the addition of potassium carbonate (3.0g) and the mixture was then heated under reflux for 3h. The solvent was evaporated *in vacuo* and the resultant solid dissolved in water (150ml) before acidification with concentrated hydrochloric acid. The precipitate formed was recrystallised from aqueous methanol: yield 1.5g (42%), m.p.220-222°C (lit.<sup>73</sup> 231-232°C),  $\delta_{\text{H}}$  7.27 (1H, dt, H-6), 7.57 (1H, dd, H-4), 7.83 (1H, dd, H-7),  $J_{6,\text{F}}=9.8$ ,  $J_{4,\text{F}}=8.4$ ,  $J_{7,\text{F}}=4.8$ ,  $J_{6,7}=7.4$ ,  $J_{4,6}=2.4$ ,  $\delta_{\text{C}}$  97.0 (C-4), 110.8 (CN), 113.9 (C-6), 123.3 (C-7), 124.2 (C-7a), 132.1 (C-3a), 135.3 (C-2), 159.0 (C-5),  $J_{5,\text{F}}=242.2$ ,  $J_{4,\text{F}}=27.0$ ,  $J_{3\text{a},\text{F}}=12.0$ ,  $J_{7,\text{F}}=3.0$ .

2-Cyano-6-fluoro-1-(3-hydroxypropoxy)benzimidazole (150)

2-Cyano-5-fluorobenzimidazole 3-oxide (149) (0.50g) was dissolved in the minimum of dried DMF (15ml), potassium carbonate (0.4g) was then added followed by 3-bromopropan-1-ol (0.4g), and stirring continued for 20h. The reaction mixture was poured into water (50ml) and extracted with dichloromethane (3x25ml), the organic extracts were combined, washed with water (2x75ml), dried over magnesium sulphate and concentrated to give a pale coloured liquid which crystallised in the freezer over a few days and was then recrystallised from toluene: yield 0.40g (58%), m.p.66-67°C. (Found: C, 56.5; H, 4.4; N, 18.1.  $\text{C}_{11}\text{H}_{10}\text{FN}_3\text{O}_2$  requires C, 56.2; H, 4.3; N, 17.9%).  $\delta_{\text{H}}$  2.01 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.67 (2H, q,  $\text{HO-CH}_2$ ), 4.64 (2H, t,  $\text{CH}_2\text{-ON}$ ), 4.77 (1H, t,  $\text{HO-CH}_2$ ), 7.35 (1H, dt, H-5), 7.77 (1H, dd, H-7), 7.93 (1H, dd, H-4),  $J_{7,\text{F}}=8.4$ ,  $J_{5,\text{F}}=11.8$ ,  $J_{5,7}=2.4$ ,  $J_{4,6}=2.4$ ,  $\delta_{\text{C}}$  30.9 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 56.8 ( $\text{CH}_2\text{-OH}$ ), 78.6 ( $\text{N-O-CH}_2$ ), 96.6 (C-7), 109.8 (CN), 114.0(C-5), 123.3 (C-4 and C-3a), 130.1 (C-7a), 134.7 (C-2), 161.5 (C-6),  $J_{6,\text{F}}=243.0$ ,  $J_{5,\text{F}}=26.0$ ,  $J_{4,\text{F}}=10.0$ .

2-Cyano-1-(3-hydroxypropoxy)-6-methylbenzimidazole (153)

2-Cyano-5-methylbenzimidazole 3-oxide (151) (0.50g) was dissolved in the minimum of dried DMF (10ml); potassium carbonate (0.4g) was then added and the mixture stirred for

1h. 3-Bromopropan-1-ol (0.40g) was then added and stirring continued for a further 18h. The reaction mixture was poured into water (50ml) and extracted with dichloromethane (3x25ml). The organic extracts were combined, washed with water (150ml), dried over magnesium sulphate and concentrated to a pale yellow liquid which crystallised in the freezer overnight to yield a yellow solid which was recrystallised from toluene: yield 0.15g (22%), m.p. 72-74°C. (Found: C, 62.35; H, 5.5; N, 18.0.  $C_{12}H_{13}N_3O_2$  requires C, 62.3; H, 5.7; N, 18.2%).  $\delta_H$  1.95 (2H, quin.,  $CH_2-CH_2-CH_2$ ), 2.52 (3H, s,  $CH_3$ ), 3.68 (2H, t,  $HO-CH_2$ ), 4.60 (2H, t,  $CH_2-ON$ ), 4.76 (1H, bs,  $HO-CH_2$ ), 7.30 (1H, dd, H-5), 7.60 (1H, d, H-7), 7.72 (1H, d, H-4),  $J_{5,7}=1.8$ ,  $J_{4,5}=8.4$ ,  $\delta_C$  22.0 ( $CH_3$ ), 31.3 ( $CH_2-CH_2-CH_2$ ), 57.3 ( $CH_2-OH$ ), 78.9 (N-O- $CH_2$ ), 108.9 (CN), 110.0 (C-3a), 121.6 (C-7), 122.3 (C-6), 127.4 (C-4), 130.5 (C-5), 137.0 (C-7a), 138.1 (C-2).

2-Cyano-1-(3-hydroxypropoxy)-6-methoxybenzimidazole (154)

2-Cyano-5-methoxybenzimidazole 3-oxide (152) (0.25g) was dissolved in the minimum of dried DMF (10ml), potassium carbonate (0.15g) was then added, the mixture stirred for 0.5h and finally 3-bromopropan-1-ol (0.15g) was added. The reaction mixture was then stirred for 18h before being poured into water (50ml) and extracted with dichloromethane (3x25ml). The organic extracts were combined, washed with water (150ml), dried over magnesium sulphate and concentrated to a pale yellow liquid which could not be crystallised from any solvent. As for compounds (136) and (146), purification was attempted on a column of silica gel but the product could not be isolated in a pure form. Due to the extreme polarity of (154), base-line impurities were always eluted with the product and evidence of these impurities is apparent with the desired product in the  $^1H$  nmr.

## Chapter 4

### Experimental

#### Diethyl 2-ethoxycarbonylmalonate (182)

To freshly distilled acetonitrile (50ml) was added anhydrous magnesium chloride (2.38g) under an atmosphere of nitrogen. Diethyl malonate (181) (7.6ml) was then added and the reaction mixture cooled to 0°C before the addition of triethylamine (14.0ml) and after 15 minutes' stirring, ethyl chloroformate (6.2ml). After the temperature had been kept at 0°C for 1.5h, the reaction mixture was allowed to heat up to room temperature and stirring continued for a further 18h. The reaction mixture was cooled in an ice bath, hydrochloric acid (5M, 30ml) added and the solution then extracted with ether (3x40ml). The organic layers were combined and concentrated to give an oil which was distilled on the Kugelrohr (b.p.70°C / 0.4mmHg) but was found to be mainly unreacted starting material containing traces of a high melting product which could not be identified.

#### Reaction of Meldrum's acid (186) with ethyl chloroformate

##### i) 5-(1-Ethoxycarbonyl-4[1H]-pyridylidene)-1,3-dioxane-4,6-dione (188)

A solution of Meldrum's acid (186) (1.0g) in anhydrous dichloromethane (25ml) was cooled to 0°C under an atmosphere of nitrogen. Anhydrous pyridine (2.7ml) was added dropwise over 10 minutes followed by the addition over 2h of a solution of freshly distilled ethyl chloroformate (0.7ml) in dichloromethane (5ml). Stirring was continued for a further 1h at 0°C followed by another 1h at room temperature. A further aliquot of dichloromethane (20ml) was added, and the solution poured into hydrochloric acid (2M, 5ml) and ice (45ml). The aqueous layer was extracted with dichloromethane (2x50ml) and the organic layers combined and washed with hydrochloric acid (2M, 2x15ml), saturated brine (25ml), dried over sodium sulphate and concentrated *in vacuo* to yield a red crystalline solid which was recrystallised from ethyl acetate:



yield 0.3g (15%), m.p.142-144°C (lit.<sup>108</sup> 147-148°C),  $\delta_H$  1.41 (3H, t,  $\text{CH}_2\text{CH}_3$ ), 1.63 (6H, s,  $2\times\text{CH}_3$ ), 4.53 (2H, q,  $\text{CH}_2\text{CH}_3$ ), 8.53 (4H, s, H-2', H-3', H-5' and H-6'),  $J_{\text{CH}_2\text{CH}_3}=7.2$ ,  $\delta_C$  14.6 ( $\text{CH}_2\text{CH}_3$ ), 27.1 ( $2\times\text{CH}_3$ ), 67.6 ( $\text{CH}_2\text{CH}_3$ ), 88.9 (C-2), 102.3 (C-5), 117.0 (C-5' and C-3'), 136.1 (C-6' and C-2'), 149.7 (C-4'), 157.2 (C=O), 164.3 (C-6 and C-4),  $m/z = 293$  ( $\text{M}^+$ ).

ii) Using triethylamine as the base

A solution of Meldrum's acid (186) (1.0g) in anhydrous dichloromethane (25ml) was cooled to 0°C under an atmosphere of nitrogen. Anhydrous triethylamine (2.4ml) was added dropwise over 10 minutes followed by the addition over 1.5h of a solution of ethyl chloroformate (0.7ml) in dichloromethane (2ml). Stirring was continued for a further 1h at 0°C followed another 1h at room temperature. The reaction mixture was diluted with dichloromethane (20ml) then poured into hydrochloric acid (2M, 5ml) and crushed ice (45ml). The mixture was extracted with dichloromethane (3x50ml) and the organic layers combined and washed with hydrochloric acid (2m, 2x25ml), and saturated brine (2x50ml), dried over magnesium sulphate and concentrated *in vacuo* to yield an orange oil.

Distillation at reduced pressure on the Kugelrohr showed no evidence of the desired product, only carbonaceous material.  $^1\text{H}$  nmr analysis of the recovered material only showed of the starting material.

Diethyl bis(hydroxymethyl)malonate (189)

Potassium bicarbonate (6.0g) suspended in formaldehyde solution (130ml, 35w/v) was mechanically stirred while being cooled at 5°C and diethyl malonate (181) (120.0g) was added dropwise over 1h. After a further 1h of stirring, the reaction mixture was poured into a saturated solution of ammonium sulphate (300ml); this was extracted with diethyl ether (2x150ml), the ether layers being separated and dried over magnesium sulphate. The clear, viscous product was then held at very low pressure (0.5 mmHg) with stirring at room temperature to aid crystallisation of the product: yield 125.8g (76%), m.p.45-47°C (lit.<sup>110</sup> 48-50°C),  $\delta_H$  1.27 (6H, t,  $\text{CH}_2\text{CH}_3$ ), 3.93 (2H, bs,  $\text{CH}_2\text{OH}$ ), 4.08 (4H, s,  $\text{CH}_2\text{OH}$ ), 4.22 (4H, q  $\text{CH}_2\text{CH}_3$ ),

$J_{\text{CH}_2, \text{CH}_3}=7.0$ ,  $\delta_{\text{C}}$  13.6 ( $\text{CH}_3$ ), 61.0 [ $(\text{HOCH}_2)_2\text{C}$ ], 61.4 and 61.5 ( $\text{CH}_2\text{OH}$  and  $\text{CH}_2\text{CH}_3$ ), 169.0 ( $\text{C}=\text{O}$ ).

#### 5,5-Bis(ethoxycarbonyl-2-isopropyl-1,3-dioxane (190))

Diethyl bis(hydroxymethyl)malonate (189) (7.9g), isobutyraldehyde (2.6g) and *p*-toluenesulphonic acid (catalytic amount) were all heated under reflux in toluene (100ml) for 6h incorporating a Dean-Stark apparatus and the exclusion of water (calcium chloride guard). After cooling, the reaction mixture was washed with sodium hydroxide (0.1M, 2x100ml) and saturated sodium chloride solution (2x100ml) and dried over magnesium sulphate. The toluene was evaporated off at reduced pressure to yield a viscous oil which was purified by distillation on the Kugelrohr: yield 6.4g (65%), b.p.122°C / 2 mmHg (lit.<sup>111</sup> 99-102°C / 0.4 mmHg°C),  $\delta_{\text{H}}$  0.90 (6H, d,  $\text{CH}_3$ ), 1.24 and 1.28 (6H, 2t,  $\text{CH}_2\text{CH}_3$ ), 1.78 [1H, m,  $(\text{CH}_3)_2\text{CH}$ ], 3.90 (2H, d, H-6 and H-4), 4.20 [5H, m,  $\text{CH}_2\text{CH}_3$  and H-2], 4.68 (2H, d, H-6 and H-4),  $J_{\text{CH}_2, \text{CH}_3}=7.7$ ,  $\delta_{\text{C}}$  14.3 ( $\text{CH}_2\text{CH}_3$ ), 17.1 ( $\text{CH}_3$ ), 32.8 [ $\text{CH}(\text{CH}_3)_2$ ], 53.8 (C-5), 62.1 and 62.2 ( $\text{CH}_2\text{CH}_3$ ), 69.6 (C-4 and C-6), 106.1 (C-2), 167.3 and 168.3 ( $\text{C}=\text{O}$ ). A 135° DEPT experiment was run to help with the assignment of the carbon signals.

#### cis- and trans-5-Ethoxycarbonyl-2-isopropyl-1,3-dioxane (191)

5,5-Bis(ethoxycarbonyl-2-isopropyl-1,3-dioxane (190) (45.0g) and sodium chloride (9.7g) were heated under reflux for 12h in a mixture of water (5.9g) and DMSO (250ml). After cooling the reaction mixture was poured into saturated brine (650ml) and extracted with ether (3x250ml), the ether layers were combined, washed with water (2x750ml) and dried over magnesium sulphate. The solvent was removed *in vacuo* to yield a brown oil which was purified by distillation at reduced pressure: yield 11.9g (36%), b.p.145°C / 5 mmHg (lit.<sup>109</sup> 75-80°C / 0.3 mmHg),  $\delta_{\text{H}}$  0.85 and 0.95 [6H, 2d,  $(\text{CH}_3)_2\text{CH}$ ], 1.20-1.39 (6H, m,  $\text{CH}_2\text{CH}_3$ ), 1.68-1.89 [2H, m,  $(\text{CH}_3)_2\text{CH}$ ], 2.30 and 2.90 (2H, 2m, H-5a/e), 3.60-4.70 (8H, m, H-4a/e, H6-a/e, H-2a/e,  $\text{CH}_2\text{CH}_3$ ),  $\delta_{\text{C}}$  14.3 and 14.4 ( $\text{CH}_3$ ), 17.0 and 17.1 ( $\text{CH}_2\text{CH}_3$ ), 36.1 and 36.4 [ $\text{CH}(\text{CH}_3)_2$ ], 39.0 and 40.4 (C-5), 60.8 and 61.0 ( $\text{CH}_2\text{CH}_3$ ), 67.0 and 67.8 (C-4 and C-6), 105.8 and 106.3 (C-2), 170.2 and 170.3 ( $\text{C}=\text{O}$ ).

cis- and trans-5-Hydroxymethyl-2-isopropyl-1,3-dioxane (192)

To a solution of lithium aluminium hydride (1.6g) in dried THF (20ml) was added a solution of cis- and trans-5-ethoxycarbonyl-2-isopropyl-1,3-dioxane (191) (11.5g) in dried THF (60ml) at such a rate as to generate gentle reflux. Cooling of the reaction mixture was required initially. Upon complete addition of the reactant, the reaction was heated under reflux for 4h. After cooling the reaction was diluted with ethyl acetate (50ml) and then extracted with ethyl acetate (3x50ml). The organic layers were combined, washed with brine (150ml) then water (3x150ml) to neutrality (pH=7) and dried over magnesium sulphate. The solvent was removed and the clear oily product purified by distillation at reduced pressure: yield 1.5g (16%), b.p.120°C / 3 mmHg<sup>111</sup>,  $\delta_H$  0.72-0.78 [6H, m, (CH<sub>3</sub>)<sub>2</sub>CH], 1.40-1.57 [1H, m, (CH<sub>3</sub>)<sub>2</sub>CH], 1.83 (2H, t, CH<sub>2</sub>OH), 1.99-2.19 and 3.13-3.30 (2H, m, H-5a/e, H-2), 3.55-4.20 (5H, m, H-4a/e, H-6a/e, CH<sub>2</sub>OH),  $\delta_C$  17.2 (CH<sub>3</sub>), 32.8 [CH(CH<sub>3</sub>)<sub>2</sub>], 37.5 (C-5), 60.9 and 62.5 (CH<sub>2</sub>OH), 67.4 and 68.9 (C-4 and C-6), 106.1 (C-2) 170.9 (C=O).

Attempted tosylation of cis- and trans-5-hydroxymethyl-2-isopropyl-1,3-dioxane (192)

*p*-Toluenesulphonyl chloride (0.7g) (recrystallised from petroleum) was added portionwise over 0.5h to a stirred solution of pyridine (5ml) cooled to 0°C. Stirring was continued for a further 0.5h before the addition of (192) (0.5g) and then continued for a further 24h. The reaction was quenched with water (25ml) and extracted with dichloromethane (3x25ml); the organic layers were combined, washed with hydrochloric acid (2M, 15ml), sodium hydroxide (0.1m, 25ml) and water (3x25ml), and then dried over magnesium sulphate. The solvent was removed under reduced pressure to yield a clear oil product.

<sup>1</sup>H nmr analysis showed only a very small degree of tosylation to have occurred and on standing a crystalline product precipitated from the oil which upon removal was found to be tosyl chloride. Further nmr analysis pointed to the isolated product being a mixture of the starting materials.

The previous (199, p133) procedure was repeated including the addition of a catalytic amount of 4-dimethylaminopyridine, but the material isolated was spectrally identical to that which was previously isolated.

#### 1-(3-Hydroxypropoxy)benzotriazole (196)

1-Hydroxybenzotriazole (5.0g) and potassium carbonate (5.5g) were stirred together in anhydrous DMF (50ml) with the exclusion of water (calcium chloride guard), a precipitate forming after 0.5h; 3-bromopropan-1-ol (5.4g) was then added, with stirring continued for 36h. The reaction mixture was poured into water (300ml) and extracted with dichloromethane (3x75ml); the organic layers were combined and washed with water (2x250ml) then dried over magnesium sulphate. The solvent was removed under reduced pressure and the product distilled under high vacuum to yield a colourless, viscous oil: yield 0.71g (10%), b.p.140°C / 0.1 mmHg,  $\delta_H$  1.96 (2H, quin.,  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 3.71 (2H, q,  $\text{CH}_2\text{-OH}$ ), 4.66 (2H, t,  $\text{CH}_2\text{-ON}$ ), 4.79 (1H, t,  $\text{CH}_2\text{-OH}$ ), 7.43 (1H, t, H-6), 7.60 (1H, t, H-5), 7.78 (1H, d, H-4), 8.03 (1H, d, H-7),  $J_{\text{CH}_3, \text{CH}_2}=6.2$ ,  $J_{\text{CH}_2, \text{OH}}=4.6$ ,  $J_{4,6}=8.4$ ,  $J_{4,5}=1.0$ ,  $J_{5,6}=6.8$ ,  $\delta_C$  31.1 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 56.9 ( $\text{CH}_2\text{-OH}$ ), 98.4 ( $\text{CH}_2\text{-ON}$ ), 109.4 (C-4), 119.9 (C-7), 125.1 (C-6), 127.2 (C-5), 128.6 (C-3a), 143.0 (C-7a).

Upon standing in the atmosphere the clear oil product darkened in colour over several days. The product was analysed by  $^1\text{H}$  and  $^{13}\text{C}$  nmr but no change appeared to have occurred and the product was redistilled to remove the discolouration.

#### 2,4,5-Trichloro-1-nitrobenzene (128)

1,2,4-Trichlorobenzene (198) (14.5g) was slowly added to fuming nitric acid (150ml) with continuous stirring at 0°C. Upon complete addition, the reaction mixture was poured into ice and water (500ml) whereupon a precipitate formed and was collected by filtration, dried in the vacuum oven and recrystallised from ethanol: yield 15.5g (76%), m.p.55-57°C (lit.<sup>117</sup> 56°C),  $\delta_H$  7.68 and 8.04 (2H, 2s, H-3 and H-6),  $\delta_C$  126.6 (C-2), 127.4 (C-6), 132.5 (C-5), 133.3 (C-3), 138.3 (C-4), 146.3 (C-1).

### 5,6-Dichloro-1-hydroxybenzotriazole (199)

2,4,5-Trichloro-1-nitrobenzene (128) (2.4g) was dissolved in hot ethanol (40ml), producing a yellow solution; hydrazine hydrate (100%, 0.7g) was then added and the reaction heated under reflux for 5h then allowed to cool. The orange precipitate which formed upon cooling was collected, dissolved in hot water (*ca.* 150ml) and precipitated with concentrated hydrochloric acid (pH=1). The precipitate was filtered and recrystallised from aqueous ethanol: yield 0.55g (25%), m.p. 200°C (violent decomp.; lit.<sup>116</sup> 210°C),  $\delta_{\text{H}}$  8.18 and 8.44 (2H, 2s, H-4 and H-7),  $\delta_{\text{C}}$  111.9 (C-7), 121.2 (C-4), 127.5 (C-5), 128.1 (C-7a), 131.3 (C-6), 141.4 (C-4a).

### 5,6-Dichloro-1-(3-hydroxypropoxy)benzotriazole (200)

5,6-Dichloro-1-hydroxybenzotriazole (199) (0.5g) and potassium carbonate (0.4g) were dissolved in anhydrous DMF (15ml) with the exclusion of water (CaCl<sub>2</sub> guard) and stirred for 0.5h after which time a precipitate had formed. 3-Bromopropan-1-ol (0.4g) was then added and stirring maintained for a further 18 h; the reaction mixture was then poured into water (50ml) and extracted with dichloromethane (3x50ml); the organic layers were combined, washed with water (2x150ml) and dried over magnesium sulphate. The dichloromethane was removed *in vacuo* and the oil obtained crystallised on standing. The product was recrystallised from diethyl ether: yield 0.2g (31%), m.p. 71-73°C. (Found: C, 41.6; H, 3.5; N, 16.2. C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub> requires C, 41.2; H, 3.5; N, 16.0%).  $\delta_{\text{H}}$  1.93 (2H, quin., CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 3.65 (2H, q, CH<sub>2</sub>-OH), 4.65 (2H, t, CH<sub>2</sub>-ON), 4.75 (1H, t, CH<sub>2</sub>-OH), 8.28 and 8.43 (2H, 2s, H-4 and H-7),  $J_{\text{CH}_3, \text{CH}_2}$ =6.2,  $J_{\text{CH}_2, \text{OH}}$ =4.6,  $\delta_{\text{C}}$  31.0 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 56.9 (CH<sub>2</sub>-OH), 79.8 (CH<sub>2</sub>-ON), 111.3 (C-4), 121.2 (C-7), 126.4 (C-5), 128.3 (C-6), 132.0 (C-3a), 141.8 (C-7a).

### Attempted synthesis of 4,5,6,7-tetrachloro-1-hydroxybenzotriazole (202)

Pentachloronitrobenzene (201) (5.0g) and hydrazine hydrate (100%, 4.5g) were heated under reflux for 2h in ethanol (50ml) and toluene (50ml). After cooling the solvent was removed and the resultant solid stirred in potassium hydroxide (5%, 100ml). The mixture was filtered and the filtrate acidified with concentrated hydrochloric acid: the precipitate was collected but was



found not to be the correct product. The precipitate was insoluble in all the solvents tried and could not be recrystallised. The solid did not melt and was presumed to be a salt.

Despite several attempts at this reaction, none of the desired product was isolated and no explanation can be offered for its failure.

#### Attempted reaction of benzofurazan *N*-oxide with nitromethane

i) Benzofurazan *N*-oxide (209) (2.0g) was dissolved in chloroform (30ml) and triethylamine (2.0g) and nitromethane (1.2g) added. The reaction was heated under reflux for 24h. After cooling the solvent was removed *in vacuo* and the resultant solid recrystallised from methanol. Analysis of the solid showed it to be a mixture of the starting material and other unknown components rather than the desired product.

The above procedure was repeated a further two times after the distillation of both the nitromethane and triethylamine and the drying of the chloroform but in each case the reaction was unsuccessful.

A slightly different procedure was then attempted for the above reaction:

ii) Benzofurazan *N*-oxide (209) (2.0g) was dissolved in ethanol (50ml) and the solution saturated with ammonia gas over 2h. Nitromethane (1.2g) was then added prompting a change in the solution colour from yellow to red, and stirring was then continued for a further 3h. The solvent was removed *in vacuo* and the resultant solid recrystallised from methanol to yield a yellow product. <sup>1</sup>H nmr and mass spectral analysis of the solid showed it to be unreacted starting material.

#### 3,4-Dichloroacetanilide (212)

3,4-Dichloroaniline (211) (15.0g), pyridine (12.0ml), acetic anhydride (18.8g) and toluene (500ml) were heated under reflux for 2h then allowed to cool. The solvents were removed *in vacuo*. The crude solid obtained was dissolved in hot aqueous ethanol and allowed to cool; the product was collected by filtration and dried. Yield 9.8g (52%), m.p. 120-121°C (lit.<sup>117</sup> 121°C),  $\delta_{\text{H}}$  2.07 (3H, s, CH<sub>3</sub>), 7.49 (1H, dd, H-6), 7.52 (1H, d, H-5), 8.00 (1H, d, H-2), 10.13 (1H, s, NH),  $J_{5,6}=7.2$ ,  $J_{2,6}=2.4$ . A D<sub>2</sub>O shake collapses the signal at  $\delta$  10.13.  $\delta_{\text{C}}$  23.9

(CH<sub>3</sub>), 118.9 (C-6), 120.2 (C-2), 124.4 (C-4), 130.4 (C-5), 130.9 (C-3), 139.3 (C-1), 168.7 (C=O),  $m/z$  = 203 / 205 / 207 (M<sup>+</sup>·).

#### 4,5-Dichloro-2-nitroacetanilide (213)

To 3,4-dichloroacetanilide (212) (26.0g), was added glacial acetic acid (17.5ml) followed by concentrated sulphuric acid (34.3g) and the solution stirred and cooled to 0°C. A cooled solution of concentrated nitric acid (d 1.40, 10.6g) and concentrated sulphuric acid (8.6g) was then added over 0.5h, stirring then being continued for a further 2h. The reaction mixture was poured on to ice (250ml) yielding a yellow precipitate which was collected by filtration and washed with water until the washings were neutral. The product was purified by column chromatography and recrystallised from ethanol to yield a yellow crystalline solid: yield 10.1g (32%), m.p. 123-124°C (lit.<sup>117</sup> 123-124°C),  $\delta_H$  2.11 (3H, s, CH<sub>3</sub>), 7.98 (1H, s, H-6), 8.25 (1H, s, H-3), 10.41 (1H, s, NH). A D<sub>2</sub>O shake collapses the signal at  $\delta$  10.41.  $\delta_C$  23.5 (CH<sub>3</sub>), 125.6 (C-6), 126.4 (C-4), 126.5 (C-3), 131.5 (C-1), 136.5 (C-5), 140.1 (C-2), 168.8 (C=O).

#### 4,5-Dichloro-2-nitroaniline (214)

4,5-Dichloro-2-nitroacetanilide (213) (0.50g) was heated under reflux in 70% sulphuric acid (3ml) for 0.75h. The reaction mixture was poured into cold water (17ml) and yielded a yellow precipitate; the mixture was basified to pH 10 with concentrated ammonia solution and the precipitate collected and washed with water (20ml) then recrystallised from ethanol and water to yield orange needles: yield 0.30g (72%), m.p. 181-183°C (lit.<sup>132</sup> 175°C),  $\delta_H$  7.26 (1H, s, H-6), 7.65 (2H, s, NH<sub>2</sub>), 8.10 (1H, s, H-3). A D<sub>2</sub>O shake collapses the signal at  $\delta$  7.65.  $\delta_C$  116.4 (C-4), 119.8 (C-6), 126.4 (C-3), 129.3 (C-2), 138.2 (C-5), 145.1 (C-1).

## **Chapter 5**

### **Experimental**

#### **5.1 Procedure**

Each of the compounds to be tested was fully characterised using  $^{13}\text{C}$  and  $^1\text{H}$  nmr spectroscopy and C/H/N analysis, and those samples which were found not to be completely pure were not tested. In the event of activity being found, any impurities in the samples used could be held responsible for the said activity; each impurity present is effectively a further compound being tested for activity against the virus used in the assay. The compounds to be tested were made up as solutions in DMSO at a concentration of *ca.* 20 mM, and immediately prior to the assay these solutions were diluted with cell medium to a maximum concentration of 200 $\mu\text{M}$ .

#### **5.2 Cells and Viruses**

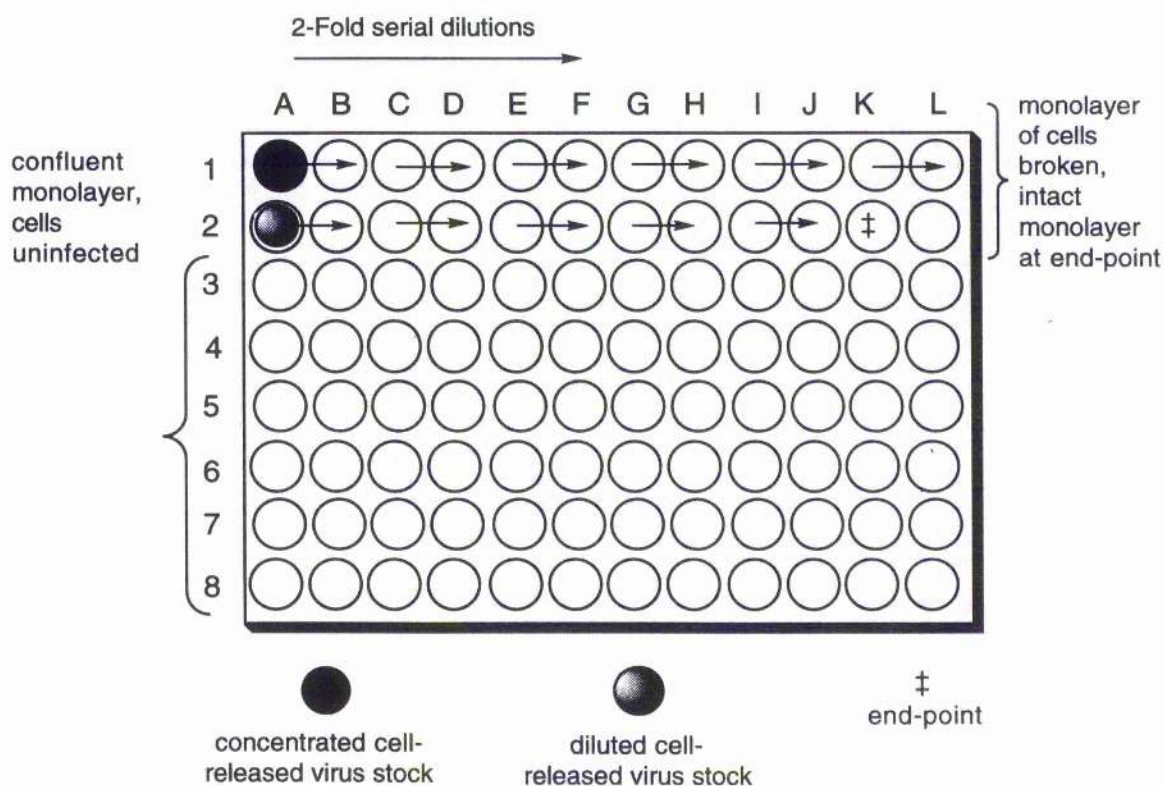
Vero cells were grown in Glasgow-modified Eagle's medium (G-MEM) (Gibco) with 10% new-born calf serum (CS). High-titre HSV1 was prepared from infected vero cells by the extraction of freeze fractured cells; the virus was stored in 50% glycerol solution at  $-70^\circ\text{C}$ .

#### **5.3 End-point titration**

An end-point titration of the virus stock was carried out to find the concentration at which the virus infected the entire monolayer over a 48h period (see overleaf).



For the titration, a stock of vero cells was removed from a stock bottle using trypsin / EDTA solution, and diluted to 40 ml with GS-MEM /10% CS. The cells were then divided and incubated at 37°C in a 96-well microtitre plate for two days, to allow a confluent monolayer to grow. The first well of the microtitre plate was infected with thawed, concentrated cell-released virus stock, and two-fold serial dilutions made across the plate. The plate was once again incubated over 48h, with checking after 24h, to establish that the cytopathic effects of the virus were not progressing too fast. After 48h an end-point was found (a point after which the monolayer remained intact; the virus had been diluted to such a level that no plaque-forming units remained, hence the intact monolayer indicated healthy cells), and so a dilution at which the monolayer was completely infected after 48 hours; 1 : 100 000 ( $10^5$ ).



### 5.3 Fixing and staining

After analysis of the monolayer of infected cells for adequate cytopathic effect (cpe), through the progression of viral cycles, the cells were fixed and stained. The cell medium was removed and replaced with a solution of formalin (2% aqueous formaldehyde) for a period of *ca.* 10 minutes. The formalin was removed and replaced by the stain, crystal violet, for a further period of 10 minutes. The stain was gently washed out with water and the cells analysed more closely for cpe or an absence due to compound activity. The second plate was analysed for evidence of toxicity shown by the cells. The fixing and staining procedure was repeated for the titration and all assays completed.

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